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CONTENTS

1912

No. 1. MARCH

J. PARSONS SCHAEFFER. The genesis and development of the nasolacrimal passages in man. Thirty-one figures.....	1
CHARLES R. ESSICK. The development of the nuclei pontis and the nucleus arcuatus in man. Twelve figures.....	2
RUSKIN M. LHAMON. The sheath of the sinoventricular bundle. Five figures	55
ALBERT KUNTZ. The development of the adrenals in the turtle. Nine figures.....	71

No. 2. MAY

DARMON A. RHINEHART. The nerves of the thyroid and parathyroid bodies. Five figures.....	91
ALEXANDER S. BEGG. The anomalous persistence in embryos of parts of the peri-intestinal rings formed by the vitelline veins. Five figures.....	103
JOHN LEWIS BREMER. The development of the aorta and aortic arches in rabbits. Nine figures	111
JEREMIAH S. FERGUSON. The behavior and relation of living connective tissue cells in the fins of fish embryos with especial reference to the histogenesis of the collagenous or white fibers. Ten figures.....	129
H. E. JORDAN AND K. B. STEELE. A comparative microscopic study of the intercalated discs of vertebrate heart muscle. Twenty-three figures.....	151
CHAS. W. GREEN. A new type of fat storing muscles in the salmon. <i>Oncorhynchus tshawytscha</i> . Two figures.....	175

J. PARSONS SCHAEFFER. Types of ostia nasolacrimalia in man and their genetic significance. Fifteen figures.....	183
FREDERICK TILNEY. The development of the veins and lymphatics in <i>Tragulus meminna</i> , <i>erleben</i> . Fourteen figures	193

No. 3. JULY

FRANKLIN P. MALL. On the development of the human heart. Thirty-seven figures.....	249
OSWALD S. LOWSLEY. The development of the human prostate gland with reference to the development of other structures at the neck of the urinary bladder. Eleven figures.....	299
ELIOT R. CLARK. Further observations on living growing lymphatics: their relation to the mesenchyme cells. Eighteen figures.....	347

No. 4. SEPTEMBER

HAL DOWNEY. The attachment of muscles to the exoskeleton in the crayfish, and the structure of the crayfish epiderm. Five figures.....	381
OTTO F. KAMPMEIER. The development of the thoracic duct in the pig. Thirty-five figures (five colored plates).....	401
FREDERIC T. LEWIS. The form of the stomach in human embryos with notes upon the nomenclature of the stomach. Twelve figures.....	477

THE GENESIS AND DEVELOPMENT OF THE NASOLACRIMAL PASSAGES IN MAN

J. PARSONS SCHAEFFER

Yale University

From the Anatomical Laboratories of Cornell and Yale

THIRTY-ONE FIGURES

A brief review of the literature on the nasolacrimal passages [lacrimal ducts (lachrymal canaliculi), lacrimal sac, nasolacrimal duct] shows that diverse views were from time to time advanced on the genesis and development of these passages. Before considering the material studied in this investigation I want to refer to some of the theories held by earlier writers. I do not wish to give a complete résumé on the history of the development, but rather in a brief manner indicate the evolution in our knowledge concerning the genesis and development of these passages.

v. Baer ('28-'37) thought that the nasolacrimal passages had their origin in a diverticulum from the 'Rachenhöhle.' His theory¹ presumably was based upon hypothetical conclusions, since it is entirely unsupported.

Burdach ('37) in his 'Die Physiologie als Erfahrungswissenschaft' writes briefly concerning the genesis of the nasolacrimal

¹ Die Bildung des Thränenkanals (in birds) glaubte ich in einer Ausstülpung der Rachenhöhle, die zuerst nur wenig vor der Eustachischen Röhre liegt und sehr bald nach dieser sichtbar wird, zu erkennen, doch habe ich bisher noch nicht den gesammten Vorgang verfolgt. Über Entwicklungsgeschichte der Thiere, Thiel 2, S. 116.

Der Thränengang stülpt sich auch hier (in mammals) aus der Rachenhöhle gegen das Auge hervor und liegt Anfangs hinter den Muscheln, die nur, indem sie sich verlängern, sich über ihn ziehen. Über Entwicklungsgeschichte der Thiere, Thiel 2, S. 219.

passages, but does not state his meaning clearly.² He apparently thought that the nasolacrimal passages had their origin in a diverticulum or skin-fold ('Hautfalte') in the region of the medial palpebral commissure (internal canthus) and, since the naso-optic fissure is obliterated by the eighth week of embryonal life, he must have thought that the 'Hautfalte' grew into the substance of the maxilla, ultimately reaching the nasal cavity. Burdach may have had the right conception of the development of the nasal end of the nasolacrimal duct, but in the genesis of the nasolacrimal passages from a skin-fold ('Hautfalte') in the region of the medial palpebral commissure he erred (providing the writer interprets his statement correctly). It is difficult to say what Burdach meant by his 'Hautfalte.' That the anlage of these passages comes to lie in the body of the maxilla is true, but it comes about in an entirely different way as will be seen subsequently. His theory would not explain the origin of the paired lacrimal ducts.

Erdl ('45) and Coste ('47-'59), according to Ewetzky, believed that the furrow "welche am Naseneingange beginnt und am inneren Augenwinkel mündet, auf ihrer ganzen Länge überbrückt und dergestalt in einem Canal verwandelt." That the naso-optic fissure becomes constricted or shut off from the surface by its lips closing in and coalescing with each other, thus establishing the nasolacrimal connections, was indeed the accepted theory for some time. The theory is, of course, erroneous because the anlage of the passages is for some time represented by a solid plug or strand of epithelial cells which early becomes detached from the surface. The strand of cells becomes cord-like and acquires a lumen secondarily (see subsequent paragraphs). Neither would this theory explain the pairing of the lacrimal ducts.

So far as my review of the literature would prove, Born ('76) was the first investigator to properly interpret the earliest stages

² Der innere Augenwinkel ist mehr verlängert als bei Erwachsenen und steht tiefer als der äussere; schon in der achten Woche erscheint in ihm die Karunkel und eine zur Mudnasenhöhle sich sekende Hautfalte als Anfang des Thränenkanals. Die Thränenpunkte ragen im fünften Monat sehr stark hervor und im siebenten etwas mehr zurück.

of the nasolacrimal passages. He investigated this field in amphibia and found a structure homologous with that described by Coste for mammals, but he found that its genesis did not agree with Coste's hypothesis.

Since Born's conception of the genesis of the nasolacrimal passages in amphibia applies also, broadly speaking, in other forms, it may not be amiss to briefly quote his own words:

Der Thränenkanal der Amphibien bildet sich durch Einwachsung und Abschnürung eines Epithelstreifens von der Nase bis zum Auge hin der dann ein Lumen bekommt und sich mit der Nasenhöhle in Verbindung setzt.

While the above did not clear up the origin of the lacrimal ducts in mammals, it nevertheless proved to be the correct interpretation of the genesis of the main portion of the nasolacrimal duct in all investigated forms up to the present. According to Born, in amphibia, a solid strand of epithelial cells, extending from the eye to the nose, becomes detached from the surface epithelium and this strand of cells later acquires a lumen. The strand of cells retains connections with the surface epithelium at both the ocular and nasal ends. This strand of cells becomes both the lacrimal ducts and the whole of the nasolacrimal duct.

Born later ('78) investigated lizards and birds and found that the basic principles concerning the anlage of the nasolacrimal passages in these forms agreed with what he found in amphibia. He, however, found that the cord of cells differentiated along the course of the oculo-nasal furrow; also that it differed somewhat in its further development. In both forms (lizards and birds) a solid cord of cells became isolated from the surface. In *lizards* the isolation was complete, i.e., there remained no connection with the surface epithelium at any point; both lacrimal ducts and the nasal end of the nasolacrimal duct developed as sprouts from the mother cord of cells. As in amphibia the lumina of these several channels were established later. In *birds* the cord of cells retained connection with the surface epithelium at both the ocular and nasal ends. One of the lacrimal ducts, however, developed as a sprout from the ocular end of the mother cord of cells.

Ewetzky ('79) studied the embryology of the nasolacrimal connections in 'Rindsembryonen' and in the main agreed with Born's findings.

Born ('82) investigated reptiles and found that the solid cord or strand of epithelial cells retained connection with the surface epithelium at the ocular end, but that the nasal end of the nasolacrimal duct grew as a sprout from the mother cord of cells. He also found that there is no doubling or pairing at the ocular end; that is, he found but one lacrimal duct.

Legal ('83) investigated the pig and came to the same general conclusion as did Born, and claimed (for pig) that the superior lacrimal duct was wholly a part of the original mother cord of cells. He further claimed that the mother cord of cells retained superiorly and dorsally a connection with the epidermis in the region of the palpebral fissure.³ He concluded that the inferior lacrimal duct grew as a sprout from the mother cord, but he found that the sprout did not reach the free border of the inferior eyelid; therefore remaining 'funktionell unbrauchbar.'⁴

Kölliker ('84) believed that both lacrimal ducts developed as sprouts from the mother cord.

Ewetzky ('88) thought that the ocular end of the mother cord divided into two forks, and that these forks in turn developed into the lacrimal ducts (superior and inferior).

Jouves ('97) studied the sheep and man, and found in a 19 mm. human embryo both lacrimal ducts present but without any connection with the surface epithelium at this time.

Cosmettatos ('98) investigated the rabbit, and Stanculeanu ('00) the bird, the sheep, and man. These investigators, accord-

³ . . . ganz hinten endlich bleibt beständig eine Verbindung mit der Lidfurche erhalten.

⁴ Bei Schweinsembryonen ist die Thränenkanalanlage eine solide, von der tiefen Epidermisschicht des Thränenfurehengrundes ins Bindegewebe einwachsende Leiste, die sich bis auf das hinterste Ende am innern Augenwinkel von der Epidermis abschnürt, und mit dem vordern, stark auswachsenden Ende mit der Nasenhöhle verbindet: der abgelöste, solide Epithelstrang stellt den spätern einfachen Thränenangang und das obere Thränenröhrchen dar, das untere sprosst aus demselben hervor, bleibt aber, da es die freie Lidfläche nicht erreicht, funktionell unbrauchbar.

ing to Fleisher, depending upon the form studied, came to the conclusion that one of the two lacrimal ducts was wholly or partly a portion of the original mother cord of cells, and that the other lacrimal duct and the remaining portion of the nasolacrimal duct developed as sprouts from the mother cord.

Hammar ('02) shows a model⁵ of a human embryo 18.5 mm. long in which both the nasal and ocular ends of the anlage of the nasolacrimal passages are free from the surface epithelium. The anlages of the lacrimal ducts are well illustrated in the model.

Fleisher ('06) published his researches on the pig, the guinea pig, the mouse, the rabbit, and man. He arrived at the following general conclusion:

Aus diesen Präparaten geht hervor, dass beim Menschen die Entwicklung der Tränenröhrchen in derselben Weise vor sich geht, wie bei den anderen, von mir untersuchten Säugetieren, durch selbständige Sprossung jedes der Röhrchen aus dem Augeneinde der Tränenleiste, die sich vollständig vom Epithel abschnürt.

Fleisher, therefore, disagrees with Legal and some others on the genesis of the lacrimal ducts (lachrymal canaliculi), and conforms with Kölliker and more nearly to Ewetzky. He is also in accord with Matys who came to similar conclusions for *Spermophilus citellus*.

Lang ('11) reports his findings in a human embryo, aged from seven to eight weeks. He finds that the left side of his embryo agrees with the conclusions of Fleisher and Matys. On the right side he, however, finds the superior lacrimal-duct anlage wanting.

In subsequent portions of this paper I wish to record my preliminary observations on the genesis and development of the nasolacrimal passages in man. I now hope to carry this study farther and, if an investigation of a larger number of human embryos warrants, will report my later observations and conclusions in a subsequent paper.

In looking over material for the substance of another paper I noticed variations in the development of some portions of the

⁵ Studien über die Entwicklung des Vorderdarms und einiger angrenzenden Organe, Archiv f. mikrosk. Anat., Bd. 59, taf. 26, fig. 14.

nasolacrimal passages. I, therefore, felt that there was need of an investigation of the genesis of these passages in man, based upon an examination of a larger number of human embryos than was formerly done. Fortunately there were available for this study good series of appropriately aged embryos showing the genesis and early stages of the nasolacrimal passages. The embryos ranged in age from thirty days to 'term.' A certain amount of material of the early extra-uterine period was also studied; together with a large number of adult specimens.

It is well known that at one stage of the embryo there is a furrow or fissure—the naso-optic fissure—extending from the eye to the nasal pit. This fissure is bounded superiorly by the lateral nasal process and inferiorly by the maxillary process. The naso-optic fissure gradually disappears by a growth and coalescence of the structures bordering it. In this manner the fissure is 'out-folded' as it were and thus becomes shallower and shallower until its ultimate obliteration. The epidermis along the course of the floor of the now very rudimentary fissure concerns us for some time longer with reference to the anlage of the nasolacrimal passages.

Before the naso-optic fissure is entirely obliterated we have in frontal sections a thickening of the deeper layers of the epidermis along the floor of the very rudimentary fissure (fig. 2). This initial thickening is the anlage of the nasolacrimal passages. It is at first, and remains so for some time, a solid cord-like structure of epidermal cells, at all points a part of the epidermis, along the floor of the remains of the naso-optic furrow, extending from the neighborhood of the eye towards the nose.

My observations began on embryos aged approximately from thirty to thirty-two days. In these embryos I could find no evidence whatever of an anlage of the nasolacrimal passages. In fig. 1 we have a frontal section through the remains of the naso-optic fissure (human embryo aged approximately thirty-three days). Note that there is no evidence of the anlage of the nasolacrimal passages along the floor of the rudimentary fissure. The epidermis appears uniform in thickness at all points, i.e., the epidermis is not thickened along the floor of the fissure.



Fig. 1 Frontal section through the nasal fossa (*f*) and the now-rudimentary naso-optic furrow (*no*), from a human embryo aged approximately thirty-three days. Note that there is no evidence of thickening of the epidermis along the floor of the rudimentary naso-optic furrow (*no*), to establish the anlage of the nasolacrimal passages. $\times 62.4$

Fig. 2 A similar section to that illustrated in fig. 1, from a human embryo aged thirty-four days. Note the plug-like thickening of the epidermis in the position of the rudimentary naso-optic furrow. This is the first evidence of the anlage of the nasolacrimal passages. $\times 62.4$.

Fig. 3 A corresponding section to those illustrated in figs. 1 and 2, from a human embryo aged approximately thirty-five days. Note the marked in-growth of the epidermal plug in comparison to the plug represented in fig. 2. $\times 62.4$.

Fig. 4 A frontal section through the anlage of the nasolacrimal passages, from a human embryo aged forty-three days. Note that the cord of epidermal cells is now entirely separated from the surface. It is wholly surrounded by mesenchymal cells. In the sections preceding and following this the separation from the surface was just as complete as in that shown here. $\times 62.4$.

The first evidences of the anlage of these passages I found in a 12 mm. embryo, aged about thirty-four days. The anlage is well illustrated in figs. 2 and 7 as a plug-like thickening (in frontal section) of the deeper layers of the epidermis along the floor of the remains of the naso-optic fissure. Note especially that the surface layer of flat epidermal cells is not included in the anlage

of the nasolacrimal passages. In this respect I am in accord with Born, Legal and others and at variance with Ewetzky's first paper ('79). As to age for the first evidences of the anlage of the nasolacrimal passages I agree rather closely with Ewetzky, who

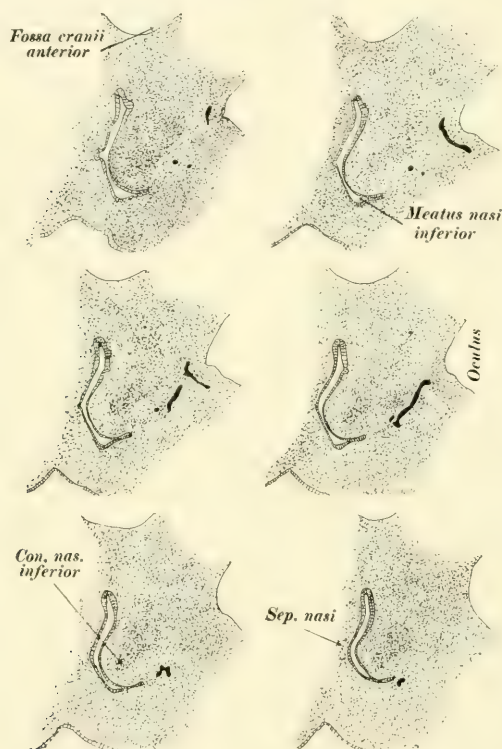


Fig. 5 Selected sections from a series through the developing nasolacrimal passages (human embryo aged from forty-three to forty-five days). Note that nowhere are the anlages of the nasolacrimal passages in connection with the surface. The lacrimal ducts are already in evidence. All of the 'passages' are yet solid cords of epithelial cells, and are indicated in deep black. $\times 14$.

found that "die Entwicklung des Thränencanals beginnt um das Ende der 5. oder im Anfang der 6. Woche des Fötallebens."

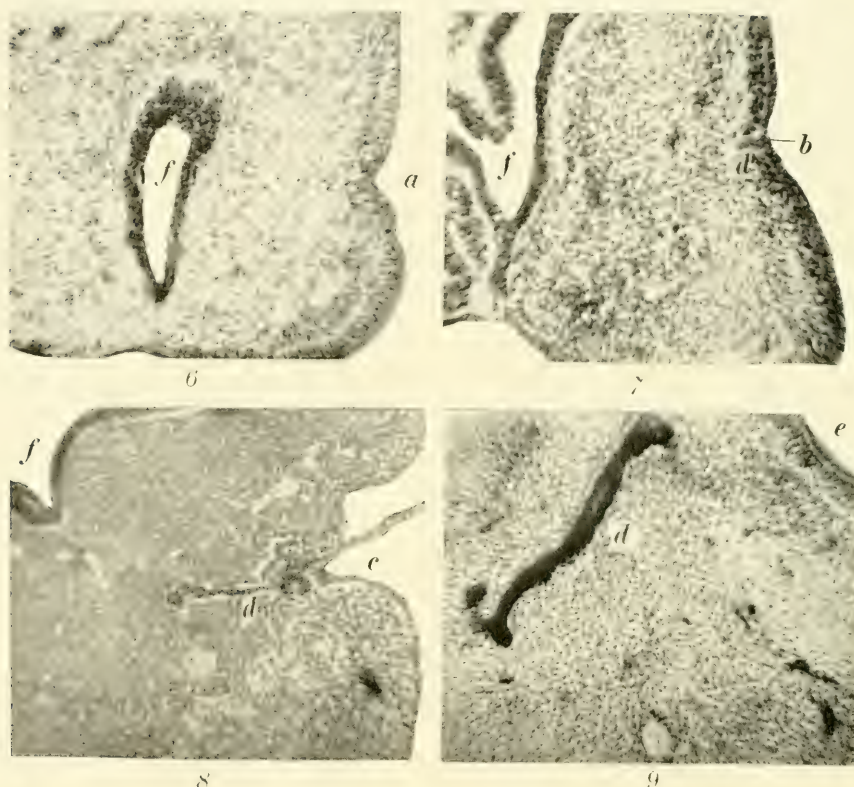
The anlage of the nasolacrimal passages soon becomes sufficiently developed to sink into the corium along the course of the rudimentary naso-optic fissure. The anlage grows rapidly

and in a brief time it has grown to such an extent that it reaches into the underlying mesenchymal tissue. Witness for example figs. 3 and 8, which represent frontal sections through the left nasal fossa of an embryo aged approximately thirty-five days. In these figures the ridge of epithelial cells has grown into the underlying mesenchymal tissue. Note especially, however, that the anlage still retains its connection with the rete mucosum of the epidermis along the course of the naso-optic groove, in which it has its genesis. Note further that the anlage is as yet solid and that there is no evidence of lumen formation.

Fig. 9 represents a semi-frontal section through a later stage of the anlage (embryo aged about forty-three days). Note that now the cord of epithelial cells is entirely detached from the surface, i.e., it has entirely lost its connection with the rete mucosum of the epidermis from which it arose. The anlage of the nasolacrimal passages now lies well embedded in the mesenchymal tissue. It will be further noticed that the central cells of the cord-like anlage (fig. 9) have taken the stain less deeply, and apparently there is already an attempt at lumen formation. Some of the central cells seem to be in a state of 'necrobiosis.' The cells of the cord are apparently becoming re-arranged to form a wall in anticipation of a later lumen.

In the serial frontal sections through the nasal cavity of a forty-three day embryo represented in figs. 10 and 11, the complete isolation from the surface of the nasolacrimal passages at this stage of development is well illustrated. The embryo from which these photomicrographs were made is in a splendid state of preservation. It is human embryo no. 3 (Hess Embryo) of the Cornell University Series. It belongs to the research collection of Professor and Mrs. Gage.

This embryo shows several very important points in connection with the development of the nasolacrimal passages: In the first place we find at this stage that the anlages of the nasolacrimal passages are nowhere connected with the epidermis, but that they are entirely encompassed by mesenchymal tissue. In the second place it will be noticed that the cords of cells are solid, with here and there evidences of lumen formation. The series



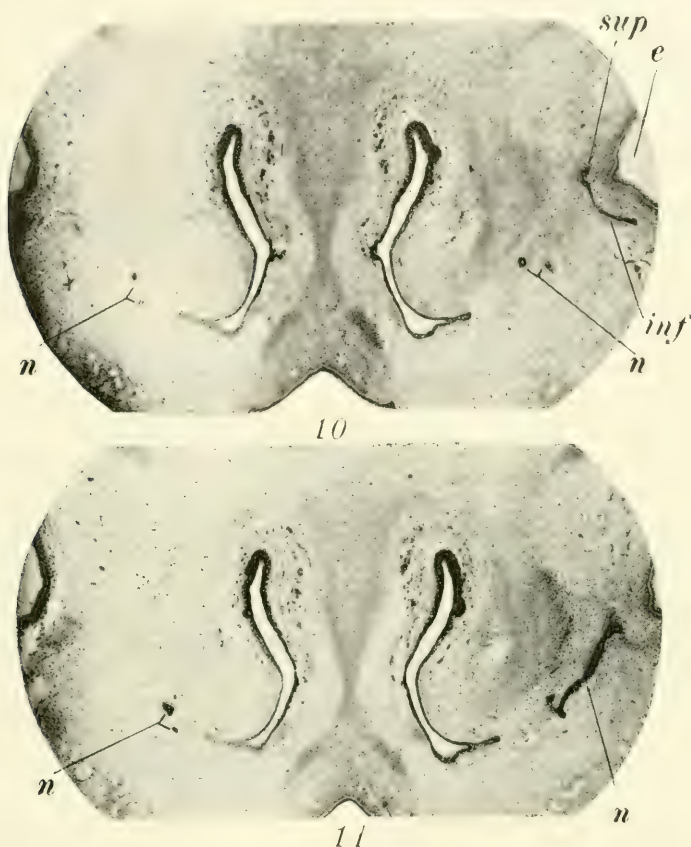
Figs. 6, 7, 8 and 9 Photomicrographs of frontal sections showing several stages in the development of the nasolacrimal passages; *a*, *b*, *c* = remains of the naso-optic furrow; *f* = nasal fossa; *e* = eye; *d* = different stages of the anlage of the nasolacrimal passages.

Fig. 6 Note that there is no evidence of the anlage of the nasolacrimal passages in the region of the naso-optic furrow (*a*). Human embryo aged thirty-three days. $\times 68$.

Fig. 7 Note the plug-like anlage of the nasolacrimal passages (*d*) from the deep layers of the epidermis along the floor of the naso-optic furrow (*b*). Human embryo aged from thirty-four to thirty-five days. $\times 68$.

Fig. 8 In this section the anlage of the nasolacrimal passages is considerably advanced over that shown in fig. 7. Note, however, notwithstanding that it has pushed its way into the underlying mesenchymal tissue, that the anlage still retains its connection with the deeper layers of the epidermis along the floor of the naso-optic furrow (*c*). Human embryo aged about thirty-six days. $\times 68$.

Fig. 9 In this section the anlage of the nasolacrimal passages has lost its connection with the surface. Human embryo aged forty-three days. $\times 68$.



Figs. 10 and 11 Photomicrographs of frontal sections in the region of the developing nasolacrimal passages, from a human embryo aged forty-three days. Note the anlage of the nasolacrimal passages and that they are entirely separated from the surface. The lacrimal-duct anlagen are well advanced and show as sprouts from the mother cord. The nasal end of the cord has not developed sufficiently to come in contact with the mucous membrane of the inferior nasal meatus. The lacrimal ducts are also some distance from the free borders of the eyelids at this time. The section represented in fig. 10 is the most ventral of the series and that represented in fig. 11 the most dorsal. Some of the intervening sections of the series are, of course, omitted. Embryo no. 3—Hess, Cornell University Series. *n* = anlage of nasolacrimal duct; *inf* = anlage of inferior lacrimal duct; *sup* = anlage of superior lacrimal duct; *e* = eye. $\times 27$.

also shows some irregularities and lateral buds from the main cords. These may account for the very common diverticula of the adult nasolacrimal ducts (fig. 30). Finally the series shows that the lacrimal ducts (lachrymal canaliculi) begin as sprouts from the ocular end of the mother cord of cells. Both the superior and inferior lacrimal ducts are about equally advanced in development, but neither of them have progressed far enough to reach the free borders of the eyelids and thus establish the definitive connections between the anlagen of the nasolacrimal passages and the epidermis.

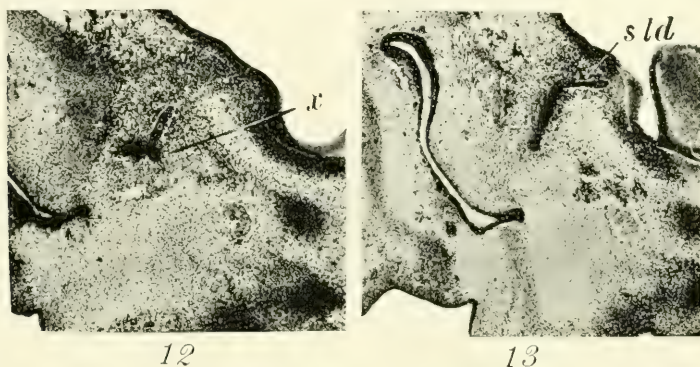
The lacrimal ducts are also solid cords of cells and show no evidence of lumen formation. In this series (figs. 10 and 11) it would indeed be difficult to say which of the lacrimal ducts (superior or inferior) was the first to begin its budding from the mother cord. Presumably they started budding approximately at the same time.

So far as my observations would prove there is considerable variation in the development of the lacrimal ducts, notwithstanding that both ducts begin, I believe, as buds from the mother cords of cells. The two ducts do not always begin their development at the same time. If they do begin at the same time then one or the other is often relatively tardy in its growth.

In figs. 12 and 13 are represented frontal sections through the nasal cavity of a forty-two to forty-five day embryo. On the left side of this embryo (figs. 12 and 13) the superior lacrimal duct is well advanced, almost reaching to the free border of the eyelid. The inferior lacrimal duct on the other hand is extremely backward in its development. The only structure present—a small lateral bud from the mother cord of cells, that may be the beginning of the inferior lacrimal duct, is shown in fig. 12. I, however, am not at all sure that this is the anlage of the inferior duct. It is a well known fact that one or the other lacrimal-duct anlage may fail to reach the free border of the eyelid. It is, therefore, possible, had this embryo (figs. 12 and 13) continued its development to 'term,' that it would have been born without a drainage duct for the inferior eyelid. On the other hand, on

the right side of the same embryo both lacrimal-duct anlagen are equally developed.

To say, from the condition found in the embryo represented in figs. 12 and 13, that the superior lacrimal-duct anlage is a portion of the original mother cord of cells is I believe erroneous. I rather hold, in such cases, that the inferior-duct anlage is tardy in its development, and that both ducts have their anlagen in buds from the mother cord. Of course as stated above one or the other duct may at times, for unknown reasons, fail to develop far enough to gain coalescence with the free border of the eyelid;



Figs. 12 and 13 Photomicrographs of frontal sections of a human embryo aged forty-two to forty-five days, in the region of the early lacrimal passages of the left side. Note that the superior lacrimal duct (*sld*) is well advanced. The only evidence of an inferior duct is seen in fig. 12, at point marked *X*. Whether this early condition would have led to an absence of the inferior lacrimal duct is of course not known. *sld* = superior lacrimal duct; *x* = anlage (?) of inferior lacrimal duct. $\times 32$.

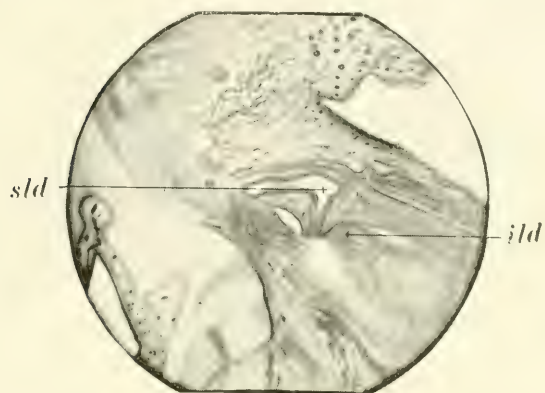
or the duct may reach the border of the lid but fail to establish a lumen at this point. Cases have also been reported in which supernumerary lacrimal puncta and lacrimal ducts were present (Weber and others). There are at times small buds arising from the lacrimal-duct anlagen, and in all probability these at times continue to develop independently until they reach and gain coalescence with the eyelids. The lumina for these supernumerary lacrimal ducts are, of course, established just as they are in the regular ducts.

By the beginning of the third month (in some cases before) the lacrimal-duct cords have developed sufficiently to come in contact with the epithelium on the free borders of the eyelids. We will, however, find that the ducts are as yet in places solid cords. Portions of the mother cord, especially the portion that is destined to become a portion of the lacrimal sac, are active in lumen formation at this time. The fundus of the lacrimal sac apparently develops as a sprout from the mother cord. The nasal end of the mother cord has not developed sufficiently to come in contact with the mucous membrane of the inferior nasal meatus. It is, however, not far removed, and in a later-stage embryo it will be found coalesced with the nasal mucous membrane. Lumen formation at the point of coalescence of the mother cord with the nasal mucous membrane is delayed approximately until 'term' (figs. 22, 23 and 24).

Frontal sections of embryos aged approximately one hundred days will show that the nasal end of the mother cord of cells has developed to the nasal mucous membrane and has coalesced with it. Both lacrimal-duct cords have grown to the free borders of the eyelids and have coalesced with the epithelium at these points. The mother cord of cells, or the portion destined to become the greater portions of the lacrimal sac and the nasolacrimal duct, has by this time established irregular lumina at various points. The latter are best developed at the ocular end of the primary cord and towards the nasal end of the cord. The lacrimal-duct cords have also established lumina at various points, especially in the regions of the knees, or what will later become the ampullae of the lacrimal ducts.

In figs. 14, 15 and 16 (embryo aged one hundred and seven days) is well illustrated the irregular manner in which the cords of epi-

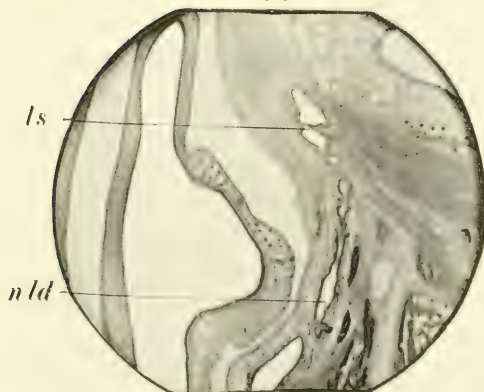
Figs. 14, 15, and 16 Photomicrographs of frontal sections through the nasolacrimal passages of a human embryo aged one hundred and seven days. Note both lacrimal ducts (fig. 14) in contact and fused with the epidermis in the region of the free borders of the eyelids. The lacrimal ducts have not yet established lumina in the regions of the eyelids (fig. 14). The remaining portions of the ducts are more or less patent throughout. Note the irregularity of lumen formation in the nasolacrimal duct (fig. 16). *sld* = superior lacrimal duct; *ild* = inferior lacrimal duct; *clld* = common lacrimal duct; *ls* = lacrimal sac; *nld* = nasolacrimal duct. $\times 10.5$.



14



15



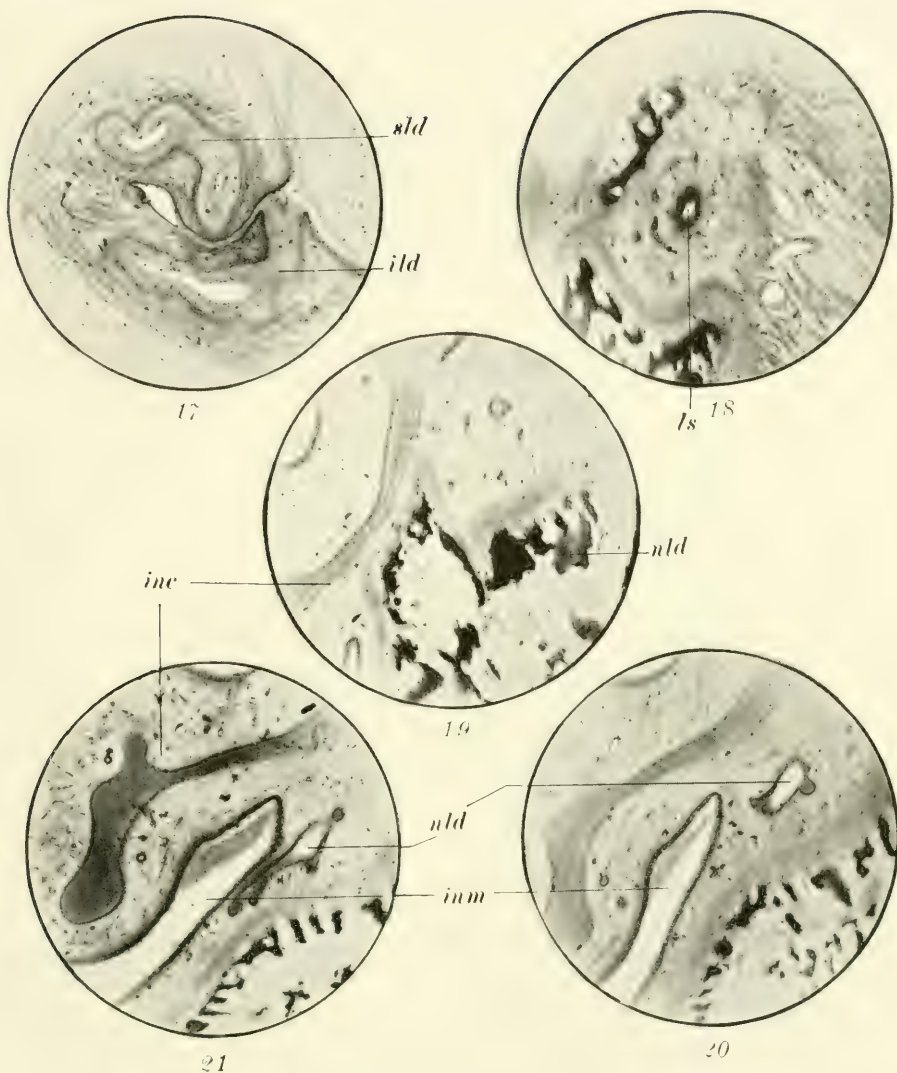
16

thelial cells establish lumina. Note that the lacrimal ducts are more or less patent throughout, save at the free borders of the eyelids where solid cords still persist. The horizontal and vertical portions of the lacrimal ducts are well shown (fig. 14). The lacrimal sac (fig. 15) is well advanced but the remainder of the nasolacrimal duct is not wholly patent. Even at this early stage there is some evidence of beginning diverticula from the nasolacrimal duct (fig. 16). The connection with the inferior nasal meatus is, of course, not yet established (fig. 16).

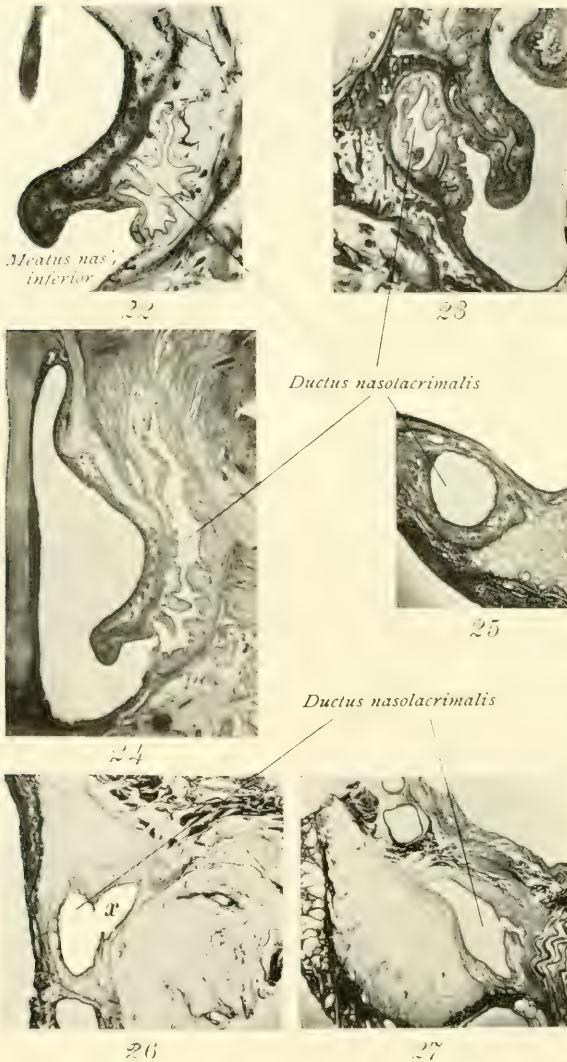
According to my studies, the ocular end of the mother cord is the first to establish a lumen. The horizontal portions of the lacrimal ducts become patent before the vertical portions (figs. 14 and 17). The last parts of the vertical portions of the lacrimal ducts to become patent are the junction points between the lacrimal-duct cords and the epithelium of the free borders of the eyelids (fig. 17). The nasal end of the mother cord establishes a lumen before the middle portion of the cord (figs. 19 and 20). The middle portion remains solid, according to my series of embryos, for some time longer (fig. 19). The last portion of the nasolacrimal passages to become patent is at the point of coalescence between the nasal sprout of the mother cord and the nasal mucous membrane. This is usually deferred, as stated before, until 'term' or even later (fig. 24).

In the adult we find varying positions on the lateral wall of the inferior nasal meatus for the ostium of the nasolacrimal duct. The ostium also varies as to shape, and it is occasionally duplicated. Rarely we find a triplicity of the ostium.

The position of the ostium, i.e., whether at the highest point of the inferior nasal meatus, or at varying distances below the above point on the lateral nasal wall, depends, of course, largely upon the original point of coalescence of the mother cord of cells with the nasal mucous membrane (fig. 28). At times the area of coalescence between the cord of cells and the nasal mucous membrane is rather extensive (fig. 21). In such cases, due to the irregular mode of lumen formation, two or more ostia may readily be formed (instead of the usual single ostium) with a bridge of intervening tissue remaining permanently. The different shapes



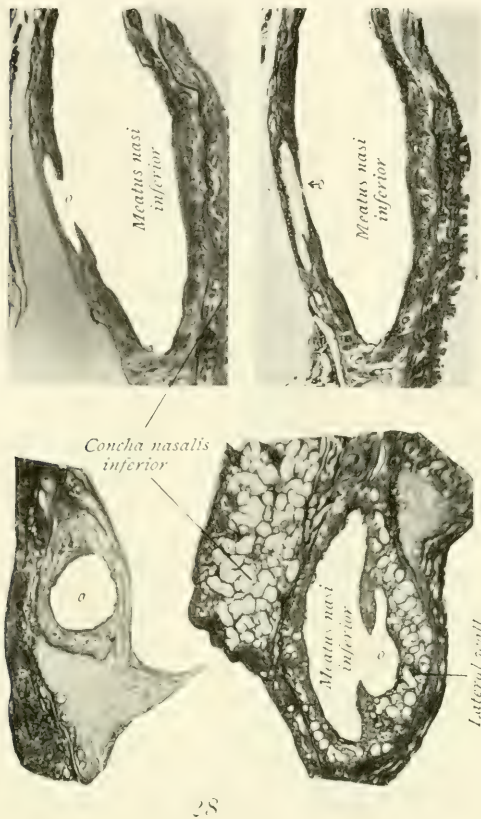
Figs. 17-21 Photomicrographs of frontal sections through the nasolacrimal passages of a human embryo aged one hundred and twenty days. Note the solid portions of the lacrimal ducts in fig. 17. In fig. 18 we have a patent section (*ls*) of the ocular end of the nasolacrimal duct, and in fig. 19 a section of the mid-portion of the nasolacrimal duct, still solid (*nld*). Note the well established lumen (*nld*) at the nasal end of the nasolacrimal duct in figs. 20 and 21. Note how extensive the contact point between the nasolacrimal duct and the inferior nasal meatus will be (fig. 21). *sld* = superior lacrimal duct; *ild* = inferior lacrimal duct; *ls* = lacrimal sac; *nld* = nasolacrimal duct; *inc* = inferior nasal concha; *inm* = inferior nasal meatus. $\times 19$.



Figs. 22-27 Photomicrographs of sections through the nasolacrimal duct.

Fig. 22 From a term child. Note that the connection between the nasolacrimal duct and the inferior nasal meatus is not yet established. $\times 3.4$.

Fig. 23 From a seven-month fetus. The connection between the nasolacrimal duct and the inferior nasal meatus is not established. $\times 6.1$.



28

Fig. 24 From a term child. Barrier between the nasolacrimal duct and the inferior nasal meatus still present. Note the irregularity of the nasolacrimal duct. Compare with fig. 30. $\times 2.9$.

Fig. 25 From an adult aged sixty years. Note the circular and regular condition of the nasolacrimal duct. Reconstruction seen in fig. 29. $\times 2.6$.

Fig. 26 From an adult aged sixty-five years. Note the marked diverticulum (X) from the nasolacrimal duct. The nasolacrimal passages of this individual are seen in reconstruction in figs. 30 and 31. $\times 2.6$.

Fig. 27 From an adult aged seventy years. In the region of the ostium of the common lacrimal duct. $\times 2.8$.

Fig. 28 Photographs of transections of the nasolacrimal duct at the point of entrance (ostium of nasolacrimal duct) into the inferior nasal meatus. Note the different types of ostia (o). The sections are from adults and are magnified from two to four times.



Fig. 29 Reconstruction of the nasolacrimal passages of an adult aged sixty years. Note the regularity of the nasolacrimal duct and compare with figs. 30 and 31. $\times 3.2$.



Figs. 30 and 31 Reconstruction of the nasolacrimal passages of an adult aged sixty-five years. Fig. 30 represents a medial view and fig. 31 a lateral view of the reconstruction. Especially note the irregularity, due to diverticula, of the nasolacrimal duct. The portions indicated in black at the inferior extremity of the nasolacrimal duct is a portion of the inferior nasal meatus. $\times 3.2$.

of the ostia are doubtless due to the angle at which the original cord of cells meets the nasal mucous membrane. The position of contact also makes a difference. If the ostium is at the highest point of the inferior meatus, i.e., just caudal to the attachment of the inferior nasal concha to the lateral nasal wall, the opening of the nasolacrimal duct is usually a large, wide, open-mouthed ostium, unguarded by folds of mucous membrane (fig. 28). If, on the other hand, the ostium is farther caudal on the lateral wall it is usually slit-like and more or less guarded by folds of mucous membrane (figs. 28).

Even at term the embryo presents very irregular nasolacrimal ducts (fig. 24). After birth the walls of the ducts become more and more regular. In the adult we very frequently find large diverticula from the nasolacrimal duct, and these added to other irregularities give us at times extremely irregular lumina (figs. 30 and 31). On the other hand we find adult ducts in which the lumina are very simple and regular (fig. 29). The lumina of the adult lacrimal ducts (lachrymal canaliculi) are generally more or less irregular.

SUMMARY

1. The strand of thickened epithelium—the anlage of the nasolacrimal passages—along the floor of the rudimentary naso-optic fissure becomes *entirely* separated from the surface, and for some time is wholly surrounded by mesenchymal tissue.

2. The strand or cord of epithelial cells thus isolated from the surface is for some time without a lumen.

3. From the mother cord of cells both lacrimal ducts and the nasal end of the nasolacrimal duct grow as sprouts. The cephalic portion of the lacrimal sac also grows as a sprout from the mother cord.

4. Considerable variation occurs in the development of the lacrimal ducts, i.e., as to number, time, and degree of development.

5. The lumina of the several portions of the nasolacrimal passages are established in an irregular manner. The ocular end

of the mother cord is the first to establish a lumen. The point of coalescence between the nasal end of the cord and the mucous membrane of the inferior nasal meatus is the last to become patent—the lumen here is established approximately at 'term' or even later. The horizontal portions of the lacrimal ducts establish lumina before the vertical portions.

I wish to take this opportunity for expressing grateful acknowledgment to Professors Gage and Kerr for the material placed at my disposal in this investigation and for other courtesies extended. I am also indebted to Professor Ferris for reading the manuscript.

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THE DEVELOPMENT OF THE NUCLEI PONTIS AND THE NUCLEUS ARCUATUS IN MAN

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TWELVE FIGURES

The following paper shows the origin of the gray matter found on the ventral surface of the adult rhombencephalon from the 'Rautenlippe' or rhombic lip, along its attachment to the medulla, and the path of migration of the cells formed here to the position characteristic of the fully developed brain. Almost all of the cells of the arcuate and pontine nuclei arise by karyokinetic division around the attachment of the roof of the fourth ventricle and then wander to their proper places. The pathway of this cellular migration proves to be a very superficial one, remarkable both for its constancy and its definite limits. The arcuate nucleus forms by a migration over the surface toward the ventral median fissure; the pontine nuclei choose a path which corresponds in every detail to the fibro-nuclear mass which I described for the adult as the corpus ponto-bulbare.

It is well known that all of the nuclear material in the central nervous system is derived from that portion of the ectoderm which closes in to form the neural tube; and our knowledge of the exact manner of this cellular distribution is due mainly to the researches of His. This author has pointed out that all of the nerve cells in the central nervous system first passed through the stage of neuroblasts and in their development are usually wont to leave the place of their origin so as to enter into the formation of the gray matter at a distance. The newly formed gray masses may: (1) remain in the neighborhood of the matrix, e.g., motor and arcuate cells of the spinal cord, or, (2) pierce the substance of the medullary tube in a radial direction and collect on the surface into an independent layer. Such wandering of cells from the

matrix of the original ventricular gray matter takes place in the formation of the cerebral cortex. (3) Subsequent to a definite bending in the medullary wall, newly formed gray masses may be transferred from the dorsal to the ventral portion of the brain by a migration of cells in a tangential direction, e.g., arcuate formations, olive and accessory olives, and a part of the nuclei lying in the pons. The third of these processes as described by His is responsible for the development of the gray matter constituting the arcuate nuclei of the medulla and the basilar nuclei of the pons.

The compact manner in which the neuroblasts arrange themselves in their migration to the pontine flexure, has attracted the attention of many observers both in macroscopic and microscopic preparations, nevertheless, with the exception of Streeter ('12) only a casual mention of it has been made by them. Blake ('00) in his description of the roof of the fourth ventricle noted cells which were transferred to the ectal surface of the oblongata by the formation of the secondary rhombic lip and he could trace them in many embryos as far cephalad as the trigeminal nerve. He ventured the suggestion that they might be connected with the ganglia of some of the cranial nerves. His ('04) has given a good illustration (fig. 103) of the rhombencephalon of a 5 cm. fetus and has shown the outlines of the thick mass of cells passing from the rhombic lip to the pontine formation. From its appearance with the naked eye, as well as in serial sections, he identifies it with the *Corpus Trapezoides*, which thus occupies a superficial position at this time (p. 163). Streeter ('07) in dissections of the Seventh nerve in pig embryos called attention to the presence of a ganglion mass connected with the pons ganglia which could be traced backward as a surface ridge between the facial and acoustic nerves, to end on the dorso-lateral surface of the restiform body. Since then he has suggested ('12) two possible origins for the pontine nuclei: the corpus ponto-bulbare and the mantle zone of the pontine region. Neuroblasts from the latter source emerge through the marginal zone as happens with the cortical cells of the cerebellum. Orzechowski ('08) in human foetus measuring 17 and 23 cm. has described ganglion masses connecting the rhombic lip, lateral recess wall and pons, which he considers the

embryonic corpus ponto-bulbare. He believes that the adult structure may contain portions of undeveloped embryonic tissue and be responsible for the frequent tumors in the cerebello-pontine angle. With the exception of the mention made by His of a migration of cells to form the arcuate nuclei nothing has been contributed to their development.

TABLE 1

LENGTH (Crown-rump)	COLLECTION NUMBER	PLANE OF SECTION	THICKNESS
<i>mm.</i>			<i>μ</i>
20	128	Coronal	50
20	368	Sagittal	20
20	22	Transverse	50
23	382	Sagittal	50
24	405	Sagittal	40
30	227	Sagittal	50
30	75	Sagittal	50
30	86	Coronal	50
33	211	Sagittal	50-100
33	145	Sagittal	50-100
35	199	Sagittal	50
46	95	Sagittal	100
50	96	Sagittal	100
50	84	Transverse	50
50	184	Sagittal	50-100
80	172	Transverse	100
96	484	Transverse	40
113	490	Transverse	30
115	219	Sagittal	50-100
143	508	Transverse	40
188	509	Transverse	50
295	491	Transverse	40

In carrying out this investigation the writer was given abundant opportunity to examine the large collection of human embryos brought together in this laboratory by Professor Mall. Table 1 gives a list of the embryos used in this study.

Wax plate reconstructions were made after the method of Born. Human, pig and rabbit embryos, stained in toto with alum-cochineal, were prepared for dissection as described by Streeter ('04, p. 87). Whole brains stained in iron-haematoxylin also gave brilliant differentiation. The most instructive specimens of

the migrating strands of nuclear material were obtained in embryos stained in methylene blue. After previous hardening (10 per cent formalin is excellent), the brain is carefully taken out of the skull and all of the pia mater dissected off; very great care must be exercised in removing this vascular membrane in order that the tiny penetrating vessels do not tear the surface of the brain which then stains very deeply along the ruptured edges. The specimen is placed into an aqueous solution of methylene blue (saturate aqueous methylene blue and water equal parts) for thirty to sixty seconds, rinsed in water, and transferred to water for study. The whole brain is tinted blue but the most prominent parts take a more intense stain so that all of the fine surface irregularities are outlined in great detail. This brings out with remarkable clearness the anastomosing strands of cells converging into the pontine formation.

Confusion might arise out of the terms employed here so that a word may not be out of place concerning their meaning. Inasmuch as the flexures of the brain as well as the position of the head are not fixed, I have used the words 'cephalad' (forward, front, anteriorly, cerebrally), 'caudad' (backward, behind, spinalward), 'dorsal,' 'ventral,' 'lateral' and 'mesial' just as if the central nervous system were a simple straight tube placed in the head as the spinal cord is in the body. This, it seems to me, will facilitate the description of relations of parts which are constantly shifting their positions in relation to the body. In addition, I might state that I have used the term 'neuroblast' loosely, so as to include all undifferentiated cells which have not taken on a definite form.

In considering the development of these basilar masses it may be of advantage to review briefly some of the relations which exist in the rhombencephalon just before the cells, destined for the pontine and arcuate nuclei, set out from their germ centers. His ('91) has carefully reconstructed some of the intramedullary nuclei and nerve roots with their relations to the surface and brain flexures in an embryo of 22 mm. (figs. 5 and 17). He has called attention to the fact that at this time, towards the end of the second month, the formation of new neuroblasts has ceased in the medulla and it is only with difficulty that a mitotic figure is dis-

covered around the ventricular cavity where great numbers were present in the earlier stages. With the cessation of its activity, the epithelium lining the cavity of the fourth ventricle becomes very sharply marked off from the underlying nervous tissue and it would be expected that the various nuclear masses in the medulla have received their allotment of cells, further growth consisting of increase in size of individual elements and the addition of nerve fibers.

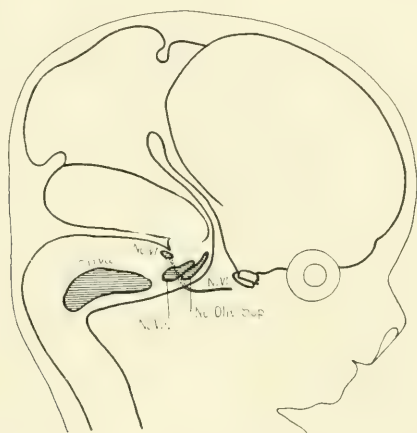


Fig. 1 Profile reconstruction of 22 mm. embryo. $\times 4.5$. Taken from His—Entw. d. mensch. Rautenhirns, fig. 5. I have drawn in the abducens nerve (N. VI) and its nucleus (Nu. VI).

If now one looks at fig. 1 (a profile drawing taken from His, ('91), to which I have added the sixth nucleus and its nerve) many differences from the adult are evident, the most striking perhaps being the great flexure in the pontine region—the cerebellar thickening almost touching the medulla. Just under the floor of the fourth ventricle appears the nucleus n. abducentis usually an elongated mass of cells lying immediately behind the ventricular furrow formed by the bend in the neural tube. From this nucleus the axones pass obliquely ventrally through the tegmentum to emerge just behind the summit of the pontine flexure in a series of rootlets which behave much as the hypoglossal nerve roots. They are quickly gathered together to form a single nerve trunk.

The facial nucleus has a remarkably constant form, the outline of which is similar both in sagittal and coronal sections. It might be compared to a pear, the smaller cephalic extremity tapering off bluntly. The nucleus preserves this constricted end in the adult as has been brought out in a model made by Mr. Weed in this laboratory. Cephalad through half of its extent it lies dorso-lateral and parallel to the superior olive and extending far in front of the outline of the nucleus n. abducentis. It will be observed that the relation to the superior olive is that of the adult yet one would miss the familiar appearance of the facial nucleus seen in transverse sections through the cephalic pole of the inferior olive. In other words, the caudal tip of the facial nucleus is distant a considerable interval from the cephalic tip of the inferior olive. The olivary complex, still very incompletely developed, is made up of an elongated mass of cells situated near the raphe. It shows a marked bend conforming to the flexure of the medulla in the neck region. Its cephalic pole, as projected on the lateral surface, falls behind the transverse level of the seventh nucleus. Of the greatest importance is the histological appearance of the rhombencephalon, the ventral surface of which is made up of the marginal veil ('Randschleier' of His) and in its nuclear free network run the fibers comprising the long association tracts. This layer, striking in sections on account of the dearth of nuclear material, forms a brilliant background which permits one to readily outline the nuclei wandering over its surface later.

From the embryological series of this laboratory definite evidences of the migration leading up to the formation of pontine nuclei appear in an embryo of 23 mm. (Mall Collection No. 382). Fig. 2 was drawn from a wax-plate reconstruction of the rhombencephalon of this embryo. Here the degree of medullary development corresponds pretty accurately to that of the 22 mm. embryo just described. The behavior of the cells lining the ventricular cavity deserves particular attention, inasmuch as they furnish the neuroblasts for the future pontine nuclei. The ependyma covering the floor of the fourth ventricle over the basal and alar plates has lost all signs of the great activity which it showed during the formation of the tegmental structures. The cells



Fig. 2 Ventro-lateral view of a wax-plate model of the rhombencephalon of a 23 mm. embryo. $\times 18$. (No. 382).

composing it are sharply marked out into a definite lamina and only after searching through many microscopic fields is one able to detect evidences of cell division. In marked contrast to this inactive region, the lip-plate which makes up the caudal wall of the lateral recess and the roof of the fourth ventricle just behind it, is found busily engaged in producing new elements. The furrow formed by the attachment of the roof plate to the medulla, contains great numbers of karyokinetic figures in every high power field of the microscope and in this neighborhood the ependymal zone is not so sharply differentiated into such a thin layer as covers the medullary floor nearer the midline. Its cells are more closely packed, its nuclei take on a deeper stain, and the line of demarcation from the subjacent tissue is partly destroyed by the protoplasmic processes of the new neuroblasts which are beginning to push toward the surface of the brain. The exact manner of arriving at the surface is illustrated by a more fortunate section (fig. 3) through a slightly older embryo. Here the deeply staining cells, poor in protoplasmic envelope, may be seen to leave their position near the ventricular cavity, and to come together at the surface where they form a tin sheet of closely arranged cellular material. When once they have gained the surface of the brain they migrate toward the pontine flexure, always preserving their superficial position.

By referring to fig. 2 a very good idea can be obtained of the zone of proliferating cells and the area covered by the migrating neuroblasts that have gained the surface of the rhombencephalon. I shall omit the description of the arcuate formation for the present and consider only that narrow elongated column of cells which is seen to turn toward the pontine flexure. It is very easy to identify the densely-staining closely-arranged nerve cells in sections and I have imitated the appearance one gets specimens stained *in toto* by shading this column. The cells, that have left the ventricle, converge into a well-defined band which, as it curves around the restiform body, embraces the more anterior of the rootlets of the glossopharyngeal nerve and passes between the facial and acoustic nerves as far forward as the trigeminal nerve. At this stage the cellular sheet is very thin, being but 4-5 cells deep

between the seventh and eighth nerves and where it ends behind the fifth nerve being but a single cell in depth.

We have then a narrow well-defined band of neuroblasts derived from germinal centers situated along the attachment of the roof of fourth ventricle to the medulla, and moving over the surface of the brain toward the pontine flexure. The histological characteristics make it possible to trace them as far as the trigeminal nerve as a sheet which gradually thins out toward its advancing edge. One might well compare the process to ice growing over a pond, yet unlike the latter the new material is formed at the shores only and the whole sheet moves out over the surface its thin advancing edge to meet its fellow from the opposite side.

It should be noted that the degree of development of the rhombencephalon does not always correspond absolutely with the measurements of the human embryos given in this table. *A priori*, it would not be expected that at any given stage each organ would always be found to correspond to those of another embryo of like measurement, but in addition to the personal elements in measuring, the fluid in which they are measured often accounts for the difference of a few millimeters more or less. Embryo No. 405 measuring 24 mm. shows a younger stage from the standpoint of pontine development than No. 382 just described. In the former embryo we can see the same active participation of the ventricular epithelium in the production of new elements and the same distribution of karyokinetic figures, yet the front ranks of advancing neuroblasts have only reached the level of the facial and acoustic nerves. This would give us a stage slightly younger than No. 382 where the advancing edge has gained the transverse level of the trigeminal nerve. Furthermore another possible error is introduced by the measurements which I have made to show the growth of the pons and they must be interpreted freely since the plane of section is rarely perfect. Obliqueness of section therefore precludes accurate comparison yet the differences are great enough to draw general conclusions.

Nos. 227, 75, and 86 (measuring 30 mm.) furnish valuable steps leading up to the fusion of the columns of the advancing neuroblasts derived from the two halves of the brain. The fre-

quency and wide distribution of the karyokinetic figures occurring around the attachment of the ventricular roof as well as the caudal wall of the lateral recess, speak for the active participation that the rhombic lip is taking in the production of pontine nuclei and as a result the roof and recess wall are thickened perceptibly. The last fetus has been sectioned transversely through the medulla and fig. 3. shows well the large production of cells along the roof attachment. It is impossible to figure dividing cells at this magnification, yet in this one section which I have illustrated, I was able to count as many as 75 karyokinetic figures immediately beneath the membrana limitans interna. A very few could be

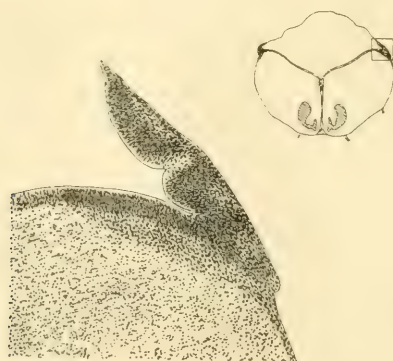
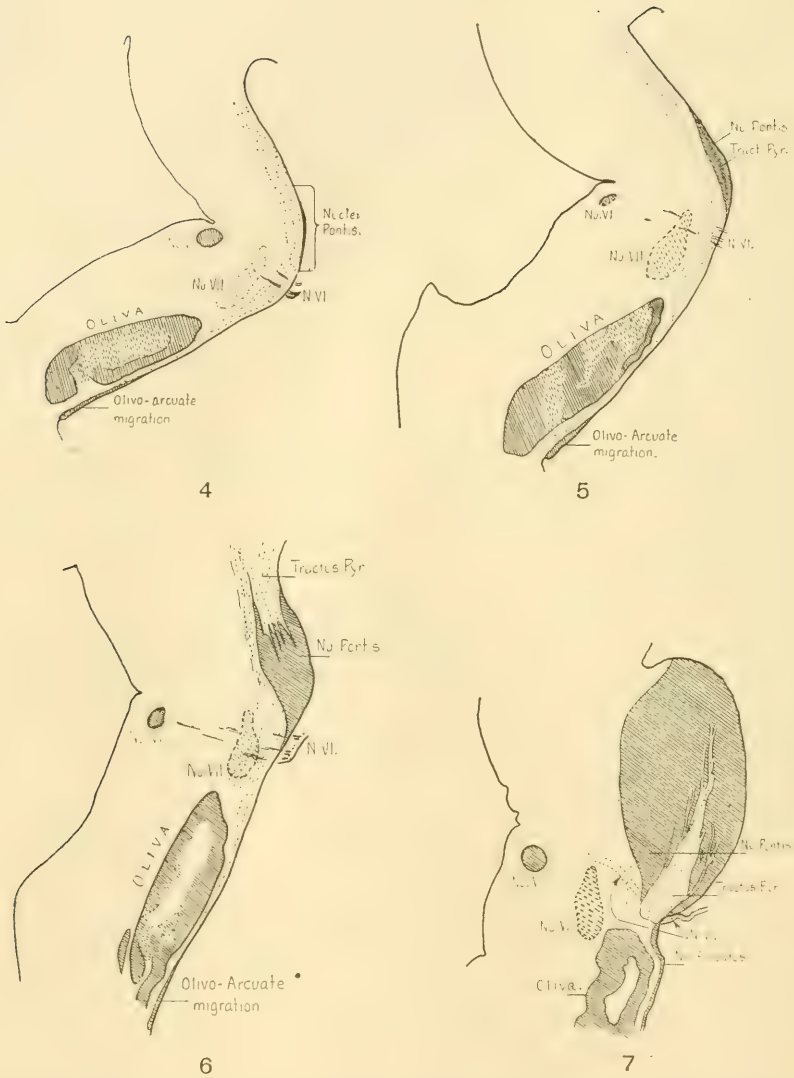


Fig. 3 Germinal centers for the basilar nuclei at the roof attachment of the fourth ventricle in a 30 mm. fetus. $\times 6.0$. (No. 86, slide 35, section 1).

made out where the cells converge at the surface, while only an occasional mitotic figure is met with among the cells turning around the restiform body. No evidences of indirect division could be found in the layer when it has arrived in front of the pontine flexure. We see, then, that the mitosis is confined sharply to the central canal. The increased production of new elements is also brought to one's attention by the increase in depth of the column passing between the facial and acoustic nerves to 10-15 superimposed neuroblasts; moreover those which had gained the trigeminal nerve in the 23 mm. embryo have moved forward and mesially in front of the pontine flexure.

The three specimens measuring 30 mm. give us the final steps in the completion of the first anlagen of the nuclei pontis. In No. 227 the cell lamina has not reached the ventral median fissure, it being possible to trace it from the border of the fourth ventricle forward to the fifth nerve where it curves mesially at almost a right angle toward its counterpart from the other side. The sagittal sections containing the sixth nerves mark the thin advancing edges of the cellular sheets approaching each other from the two sides of the brain. In No. 75 the most advanced cells have almost succeeded in gaining the midline, while in No. 86 the two columns have fused across the raphe. During their entire course from the rhombic lip on the dorsal surface to the raphe on the ventral surface, the wandering neuroblasts have kept a superficial position, only occasionally is there any tendency for any of the cells to penetrate into the clear, almost nuclear-free marginal veil. As they pass between the seventh and eighth nerves the cells are constricted into a narrow band 0.2 mm. wide, but on reaching the pontine flexure they spread out into a fan-shaped layer 0.6 mm. in caudocephalic extent. The lemnisci medialis and lateralis, which up to this stage had occupied a superficial position, are now covered over by a thin bridge of tissue and we can begin to speak of a tegmental and basilar part of the pons.

Nos. 211 and 145 (33 mm.) are cut sagittally and give us an opportunity to study the earliest pontine nuclei in their relation to the emergent nervus abducens. Fig. 4 is a camera lucida outline of the section through this nerve. The axones after leaving their nucleus take a ventro-cephalic course through the tegmentum and emerge from the neural tube just behind the most prominent part of the pontine flexure. The young pontine neuroblasts, on the other hand, lie wholly in front of this flexure, spread out into a sheet whose caudo-cephalic extent is 1.25 mm. and whose depth is 0.057 mm. at its thickest part, tapering down to the thickness of a single cell both caudally and cephalically. Between the most cephalic rootlets of the sixth nerve and the most caudal cells of the pontine nuclei is an appreciable interval (almost 0.5 mm.) so that one is at once reminded of the condition



Figs. 4 to 7 Camera lucida tracings of sagittal sections through the rhombencephalon of human embryos from Prof. Mall's collection. The nucleus facialis (Nu. VII) has been dotted in by profile reconstruction.

Fig. 4 33 mm. fetus. $\times 9.5$. (No. 145, sl. 19, sect. 3).

Fig. 5 35 mm. fetus. $\times 9.5$. (No. 199, sl. 37, sect. 1).

Fig. 6 50 mm. fetus. $\times 7.2$. (No. 96, slide 48).

Fig. 7 115 mm. fetus. $\times 7.7$. (No. 219, sl. 40, sect. 4).

seen in the adult lower animals where the abducens nerve emerges from the brain some distance behind the pontine formation.

At 35 mm. (No. 199) the increased cellular activity around the wall of the fourth ventricle is shown by the great numbers of dividing cells and the twofold increase in depth of the migrating column passing between the facial and acoustic nerves. Already the neuroblasts which are crowding from both sides toward the midline have piled up over the ventral surface of the brain, so that, near the raphe, they are now four times (0.22 mm.) as deep as the stage preceding. The free interval between the emergent abducens and the caudal edge of the pons is decreased to half what it was in the 33 mm. embryos. The important contribution which this embryo adds to the development of the basilar part of the pons, consists in a few strands of longitudinal fibers lying near the midline within the thin sheet of superficial neuroblasts newly descended from the lateral walls of the ventricle. Extending in a direction parallel to the axis of the central nervous system, these inconspicuous fiber bundles separate from the well developed bundle of axones comprising the lemniscus medialis at the level of the cephalic edge of the pontine sheet and plunge into the latter where they take up a middle position as far as its caudal edge. Here again they leave the nuclei pontis and join the fiber mass constituting the medial lemniscus. It is impossible to trace these isolated fibers except where they lie among the pontine nuclei, but, as we shall see when, by continual addition to their number, more of their course can be determined, these few strands represent the first beginnings of the longitudinal fibers which are recognized in the basilar part of the adult pons as the cortical projection system. They are represented in fig. 5 by two dotted lines traversing the pontine nuclei. Concerning the first appearance of the pyramidal tract there has been a general unanimity of opinion, the most important work being that of Flechsig's work on myelinization time. Tiedemann ('16) thought he saw pyramids in fetus of the third month but he was evidently looking at the eminences formed by the inferior olives which at this time lie adjacent to the ventral median fissure and cause an elevation in the position occupied by the future pyramidal tract.

The latter subsequently forces its way between the olives and gradually displaces them laterally. Certainly at this time the number of fibers making up the pyramidal tract is insufficient to cause the surface markings on the medulla which we know as pyramids. Reasoning back from the interval of time—four months—between the appearance and myelinization of other systems, Flechsig came to the conclusion that the pyramids must first be laid down between the middle and the end of the fifth month. In cross sections through the olive of an 80 mm.¹ (crown-rump) fetus he is unable to recognize any tissue which may be regarded as matrix for the pyramids but thinks they arise from fibers growing down from the cerebral cortex with remarkable rapidity in the second half of the fifth month. W. His ('04) has given a valuable table (p. 155) showing the various fiber systems which he was able to identify at each stage of embryonic growth. In a fetus of 83 mm. he was unable to find the pyramidal tract, but at a length of 120 mm. he saw evidences of its appearance together with cross pontine fibers. He gives us no statement as to the part of the brain in which he observed the pyramidal tract, merely noting its presence or absence in the various embryos in his collection. With this statement of the present knowledge of the cortical projection system, I shall omit until later the reasons for believing that the few axones isolated by the early pontine nuclei represent the anlage of the cortico-spinal tract in this 35 mm. fetus.

No. 95 (46 mm.) is the youngest stage in which I could determine cross fibers among the pontine nuclei. They are most conspicuous at the lateral borders where they gather together into compact strands to form the brachia pontis. Here the fibers have a superficial position, embracing laterally the corpora restiformia as the latter turn sharply into the cerebellar hemispheres. It is possible to trace the axones coming from the pontine nuclei for some distance into the cerebellum until their course parallels the

¹ The crown heel measurement which Flechsig used was 11 centimeters. For the sake of ready comparison with my study I have put his measurements into this form from the table given by Mall in *Handbuch der Entwicklungsgeschichte des Menschen-Keibel und Mall*, Leipzig, 1910, p. 205.

large mass of fibers coming up in the inferior peduncle. The trigeminal nerve in this stage sends its rootlets through these cross fibers in an oblique direction to reach its intramedullary nuclei. The cerebro-spinal neurones which were seen among the relatively thin sheet of nuclei covering the ventral surface of the pontine flexure in the stage just described (35 mm.), are increased enormously during the interval left in this series, and we have a striking similarity to the picture one gets in sagittal sections through the pontine region of the adult brain. The thickened layer of cells (now 0.642 mm.) are invaded by large anastomosing strands of fibers which collect at the cephalic and caudal border of the nuclear sheet into a solid bundle. This behavior is one of the peculiarities of the cortical projection system as it lies among the pontine nuclei where, as is well known, the otherwise compact fiber tract is broken up into smaller fasciculi by the cross fibers and nuclei of the pons. Caudad the reunited fibers fuse inseparably with the median lemniscus soon after leaving the nuclei pontis; cephalad I am unable to trace them beyond the cephalic flexure.

His has suggested that this interweaving of cross fibers of pons with pyramidal tract points to an alternating time of deposition of the component parts of the two systems—the development proceeding in a direction away from the central canal. This for the most part is true. We find the new cells which have migrated from the ventricular walls, spreading themselves over the surface of those already descended to the pontine flexure, and as the new axones come from the cortex they tend to grow among the younger nuclei, i.e., to grow nearer the surface. Thus each fasciculus when it enters the pontine nuclei, pushes along near the surface but it is soon deeply buried by new cells which are continually streaming down from the ventricle. As a result there is a separation of the pyramidal tract into a series of fasciculi which unite again at their exit from the caudal border of the pons. Some of the cells, however, after passing between the seventh and eighth nerves forsake their superficial position and plunge between the cross fibers of the pons. This is well illustrated in fig. 9 the more deeply staining young cells are seen forcing their way between

the transverse pontine fibers going into the brachium pontis. Moreover the fasciculi of the pyramidal tract keep on growing so that one must infer that some of the axones coming down from the cortex add themselves to the bundles more deeply placed in the pons.

During the period of growth between 35 mm. and 46 mm. enormous numbers of neuroblasts have come down from the lateral boundaries of the fourth ventricle. These new cells together with their processes sent out transversely and the cortical axones threading their way among them have increased the thickness of the basilar portion to 0.642 mm. There is a tendency for the cells from both sides to crowd toward the midline, thus giving rise to the typical crescentic shape of the pontine nuclei which one obtains in transverse sections through the pons. The increase in thickness is also accompanied by an increase in caudo-cephalic extent. The latter, however, does not proceed with the same proportional rate as the former, so that the sagittal sections of pontine nuclei are becoming more and more oval. In spreading caudad the interval between the nuclei pontis and the abducens rootlets has been gradually reduced until in this fetus the more cephalic axones are surrounded by pontine nerve cells.

In fetus of 50 mm. (Nos. 84, 96, 184) the number of cerebrospinal neurones have increased to such an extent that it is now possible to follow them with sufficient accuracy to be certain that we are dealing with the axones of the cortical projection system. The fibers splitting up among the pontine nuclei already form comparatively large bundles (fig. 6) which are collected together into a solid fasciculus at the cephalic end of the pons. Here they come into close relationship with the lemniscus medialis, but it is not impossible to trace the large fiber mass into the internal capsule. Traced cerebrally the crura gradually diverge from the midline and turning around the cephalic flexure they lie ventral and lateral to the nucleus hypothalamicus, while the medial lemniscus has a more dorso-lateral position with regard to this nucleus. The fibers making up the pyramidal tract can be traced definitely into the internal capsule. Spinal-ward I have been unable to differentiate the projection system from the medial lem-

niscus soon after it has left the caudal border of the pons. There is then no question but that we have been dealing with the beginnings of the pyramidal tract as early as 35 mm.; its behavior among the pontine nuclei making identification certain. Former observers have confined most of their attention to the medulla oblongata where it is hopeless to try to pick out the few strands of fibers when they first grow down from the cortex. The increase in number of these axones is so gradual that it is only in the older fetus where enough fibers are collected to form the surface marking on the medulla which we can recognize as pyramids. Flechsig is sure that there are no pyramids at 80 mm. and probably "the pyramidal tract is completely lacking." To harmonize the system with other observed facts he assumes that they must grow down rather rapidly from the cortex when once they start, since their myelinization occurs after birth and the usual interval between the formation of a nerve fiber and its acquirement of a myelin sheath is about four months. This of necessity would have the pyramidal tract appear about the middle to the end of the fifth month or 14 to 16 cm. To this one must answer that a myelin sheath does not appear on every axone of this system simultaneously; it begins rather on isolated fibers and is first complete at the age of two years.

In these fetus of the eleventh week the basilar part of the pons has reached a thickness of 0.7 mm. (fig. 6). The abducens nerve rootlets are almost entirely surrounded by nuclear material after they leave the tegmentum, only the caudal two or three fasciculi being free. Great numbers of neuroblasts are encountered passing between the seventh and eighth nerves, forming a stream 0.16 mm. deep, while the germ centers at the ventricular margin are busily producing new cells. A fortunate sagittal section through No. 96 has been illustrated to show the participation which the greatly thickened lateral recess wall takes in contributing cells to the pons. For purposes of orientation a wax-plate reconstruction was made with the section drawn on its cut surface (fig. 8). As the cerebellum in its growth crowds against the medulla, this caudal wall is flattened out and becomes part of the mesial wall of the recess. A separation of the cells coming from

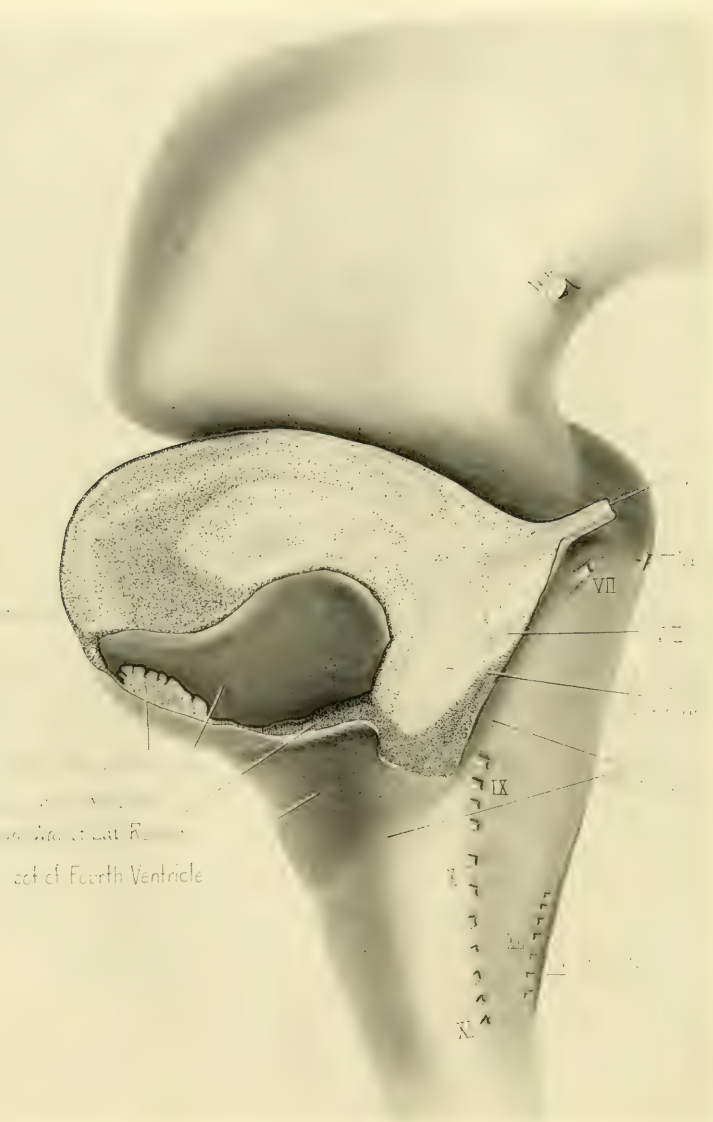


Fig. 8 Sagittal section through the cerebellum and lateral recess of a 50 mm. fetus from a wax-plate reconstruction. $\times 15$. (No. 96).

the recess wall from those of the ventricular roof is purely arbitrary, since the two origins are really continuous with each other. This association in the adult was first pointed out by Orzechowski ('08, p. 41). For embryological reasons the nuclear thickenings of the lateral recess wall with their accompanying fibers occurring in the adult should be included in the structure which I have termed the corpus ponto-bulbare. At this stage the cavity of the fourth ventricle shows a peculiar tendency to form small outpouchings along the attachment of the roof at the place where the pontine nuclei are being formed. From two to four such recesses can be made out extending laterally for a considerable distance from the main ventricular cavity and causing the external surface to be thrown up into ridges. In section they may be round or slit-like and are lined with deeply staining cells, great numbers of which are found in process of karyokinetic division. The production of neuroblasts at this stage is enormous and these lateral extensions from the ventricle furnish a greater expansion of ependymal surface and thus increase the germ layer where cell division can take place.

In the older fetus the system of ventricular outpouchings becomes more complicated and secondary processes are formed which are distinctly tubular. The size of the lumen varies, being sometimes less than the width of a single nucleus. It is always lined with a simple layer of cells which are definitely ependymal and as long as pontine nerve cells are being formed these tubules can be made out with a little difficulty among the closely packed neuroblasts but always the center of mitotic activity. With the emigration of the last of the new elements the ventricular prolongations stand out with much greater clearness. This is particularly well shown in the five and eight months fetus—in the latter, one is struck by the greater number of such tubules both in the roof attachment and the caudal wall of the lateral recess.

At the beginning of the fourth month, as shown by No. 172 (80 mm.), the evidences of marked cellular activity, i.e., extensive mitosis and deeper staining are still present around the roof attachment of the fourth ventricle and the caudal wall of the lateral recess. This fetus does not illustrate any new principle

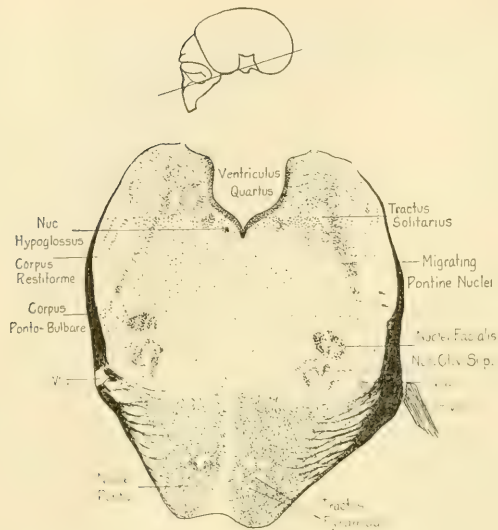


Fig. 9 Oblique section through the rhombencephalon of an 80 mm. fetus. $\times 7.5$ (No. 172, sl. 200, sect. 2).

but furnishes a beautiful single section which includes all of the relations of the cells wandering to the pontine nuclei. Owing to the cervical flexure a cut which sections the spinal cord transversely passes tangentially through the medulla and pons (fig. 9). The great thickness at which these sections were cut gives the migrating column of nuclei a very deep red stain, almost black, and I have represented it as black; the plane of section falls behind the roof thickening which marks the true germinal centers, but it does show with remarkable clearness the whole path of the neuroblasts starting from the ventricular edge, encircling the corpus restiforme, passing between the facial and acoustic nerves, to take their place among the cross fibers of the pons.

During the period extending through fetus 508 (143 mm.) there is a continued addition of cells to the basilar part of the pons. In a fetus of the thirteenth week, 96 mm. (No. 484), the maximum production of new cells has been reached. At this time the ventricular edges on both sides of the brain are full of karyokinetic figures and extending from these places are two thick columns of

closely packed young cells which pour into the pons between the seventh and eighth nerves. The cells which had already descended from the rhombic lip show no tendency to assume the ganglionic form. They are still rather closely arranged, the cytoplasm scanty and clear, the nucleus small and containing one or two chromatin condensations but no real nucleolus. Two different reactions toward the haematoxylin can be made out among the nuclei of these cells—the one quite densely staining and usually smaller nucleus, the other slightly larger and more vesicular. The latter form the larger proportion of nuclei. From the extensive cross-fiber system already present one must assume that great numbers of these pontine cells have sent out nerve processes and have taken up their final position. The newly added cells tend to remain superficially yet a considerable portion push in between the transverse fibers and cells already fixed. Although the stain of No. 490 (113 mm.) does not permit of good histological study, yet it may be readily seen that the pontine nuclei are still receiving great numbers of new elements from the rhombic lip. At 143 mm. (No. 508) the production of neuroblasts destined for the basilar parts of the hind brain has diminished very appreciably. The mitoses around the ventricular margin are fewer and the ependymal lining has begun to be separated quite sharply from the underlying nervous tissue. Many of the cells in the path of migration have larger and clearer protoplasmic bodies, apparently unwilling to complete their journey to the pontine region. Others appear as all of those in earlier stages with elongated almost naked nucleus pushing on toward the pons before assuming the ganglionic form. Of the cells which have long since gained their permanent position in the pons, many can now be recognized as ganglion cells. The nucleus is very much larger, although rarely showing a distinct nucleolus—the cytoplasm, paler than the framework in which it is embedded, is also increased in amount. The greater number of cells have grown slightly—possessing a smaller, more densely staining nucleus surrounded by a clear protoplasmic envelope.

During the interval between 143 mm. (No. 508) and 188 mm. (No. 509) the formation of neuroblasts ceases entirely and the

further development of the pons consists of an addition of axones (with their later myelinization) and the maturity of the nerve cells. The rhombic lip has given up all signs of activity in the next stage of which I had access to serial sections. In No. 509 (188 mm.) the lining of the central canal is uniformly at rest in the medullary region and is now as sharply demarcated around the roof attachment as that covering the basal and alar plates. The roof thickening now reminds one of those sections through the adult medulla which pass through the ponticulus of Henle. Comparatively few of the cells have not descended into the pontine region but are taking on the characters of adult ganglion cells along the path where the pontine cells migrated at an earlier period. Just how many cells fail to move into the pontine region but take up their position around the restiform body varies in the different brains. This helps us to understand the wide differences which were noted in the size of the fully developed corpus pontobulbare. With the disappearance of the closely packed nuclei around the attachment of the rhombic lip and the consequent clearing up of the roof thickening, the ventricular outpouchings stand out with great clearness. They are not very unlike tubular glands with a single cell lining them and show a tendency to branch frequently.

The increase in the number and size of the bundles of cross fibers in the pons gives the basilar part a greater thickness in the adult where one finds the fiber material outweighing the nuclear material. In the first beginnings on the contrary just the reverse holds true, and the nuclei pontis alone form the protuberances in the pontine region. The individual ganglion cells, although larger than the preceding stages, are still immature. The protoplasmic bodies do not accept the counter stain and few of the nuclei possess a well formed nucleolus.

By the eighth month (No. 491) the protoplasm of the ganglionic cell is no longer clear but takes up the counter stain. Many of them now look like the ganglion cells of the adult except for their smaller size. The entire migratory path is strewn with cells. Passing between the facial and acoustic nerves the column can be traced around the restiform body into the roof of the fourth ven-

tricle and the mesial wall of the lateral recess. A great many of the cells can be recognized as ganglion cells of the corpus pontobulbare, but as a whole the appearance is one of immaturity. Here and there can be found a cell whose protoplasm stains but as a rule the large vesicular nuclei are surrounded by a colorless zone. The migration of nuclear material in the medulla has ceased entirely in this stage and one has to expect only the maturity of those elements already present.

Throughout the description I have disregarded a very important factor in development which transforms the hind brain of an embryo of the second month into the adult form: I refer to the obliteration of the pontine flexure. The maximum flexure in the neural tube occurs about the time of the appearance of the first nerve cells on the ventral surface and then diminishes gradually so that at birth there is still an indication of it on the ventricular floor in front of the emmentia abducentis by a furrow running transversely. The relations of the tegmental nuclei to one another as well as to the olivary complex are distorted by the extreme flexion of the brain in the pontine region. The reduction of this may be regarded as taking place around the nucleus n. abducentis as an axis, since it is situated immediately beneath the ventricular floor just behind the bend in the brain. The other nuclei, superior olive, facial nucleus and olivary complex are distributed around its circumference and are consequently separated from one another. A glance at fig. 1 will show this arrangement. Figs. 4, 5, 6, and 7, are camera lucida drawings of sections through the nucleus n. abducentis with its emergent root bundles, which were selected from such sagittal series as illustrated the change in position of the nuclear masses during the obliteration of the pontine flexure. The nucleus facialis is projected into the section as indicated by the broken lines. As the neural tube unbends, the olivary complex and pontine nuclei are gradually pushed toward one another until the cephalic tip of the former comes to be covered by the latter. The abducens nerve which in younger stages (figs. 4, 5 and 6) pursues a straight course within the medulla is bent by this process, so that it takes a caudal direction in order to reach the surface of the brain (fig. 7). The facial nu-

cleus at first separated from the olivary body by a considerable interval comes to lie in the same transverse section as the latter, while the pontine nuclei cover up the cephalic two-thirds of this nucleus. We have, then, in addition to the mere increase in size of the pontine and olivary nuclei an actual alteration of their positions as a result of the straightening out of the neural tube. As a consequence nuclear masses which were separated from one another, are crowded together and the course of the sixth cranial nerve altered.

Having considered the origin of the main mass of nuclei pontis, the possibility of cells from other sources must not be overlooked. In the region of the pontine flexure near the raphe one can make out at an early period collections of cells extending from the ventricular floor to the pontine nuclei with which they are connected. They occupy the position which is held by the nuclei reticularis tegmenti pontis (Flechsig) in the adult. Long before any cells appeared superficially on the pontine flexure the karyokinetic figures had disappeared in the ependymal sheet near the raphe, so that it is highly improbable that the nuclei pontis depends on this portion of the neural tube for many of its elements. In addition these cells of the nuclei reticularis tegmenti pontis are evident long before the pontine nuclei appear and never have the characteristic appearance of young wandering neuroblasts during pontine development. In some of the older embryos a thin sheet (one to two cells deep) are migrating from the wall of the lateral recess in front of the dorsal cochlear nuclei but the layer is narrow and composed of comparatively few cells. These cells join the pontine nuclei behind the trigeminal nerve. It is hardly necessary to exclude other sources if one considers seriously the great production of new cells around the rhombic lip. This begins at 23 mm. and continues incessantly until the fetus has passed 143 mm. in crown-rump measurement. Couple with this extensive period the short time in which any mitotic division is complete and the great numbers met with in every section and it will not take a great stretch of imagination to account for all of the cells in the nuclei pontis.

ARCUATE NUCLEI

Examination of different adult brains in microscopical sections reveals a great variation in the amount of nuclear material which goes to make up the basilar portions of the brain stem. This is especially true of the arcuate nuclei where small, more or less isolated patches of nuclear material may often be scattered along the ventral and lateral surfaces of the medulla as far as the restiform body. The arcuate nucleus proper, the most constant of these masses, lies near the ventral median fissure superficial to the pyramidal tract, extending from a point caudal to the olive up to and fusing with the pontine nuclei. At its caudal extremity, under the olive, this mass is always of greater dimensions and tapers off somewhat as it is followed toward the pons—in some brains disappearing here and there for a few sections, in others forming a continuous narrow strip under the whole length of the medulla. The arcuate nuclei proper, as well as these superficial isolated masses lying more laterally, will be shown to have a common origin and at one time to be actually continuous with one another. The principles governing their development are identical with those which we have studied in connection with the pontine formation. The same germ centers around the attachment of the rhombic lip contribute cells which migrate superficially over the medulla in front of the cervical flexure.

The formation of the arcuate nucleus, unfortunately, is not so simple as that of the pontine nuclei, but is complicated by the simultaneous development of the olivary complex. As His has shown, the latter begins as a migration from the alar plate of the rhombencephalon early in the second month. Toward the end of this month the olive can be outlined readily although it has only a small fraction of the cells which it contains in the adult. At this time one can make out in embryos of about 20 mm. large elongated nuclei, almost devoid of a protoplasmic body leaving the ventricular margin along the attachment of the rhombic lip. They are arranged in strands of a single cell in depth and two or three in width, streaming over the surface of the medulla just under the external limiting membrane. This migration is directed

toward the portion of the medulla which is under the partially formed olivary complex and recalls the undifferentiated wandering cells seen in connection with the nuclei pontis. A great many leave the surface at various points and plunge into the depth to join the neuroblasts already massed up in the olivary nuclei. A broad sheet, however, remains superficially and can be traced from the roof attachment ventrally. In some brains (Nos. 368 and 453) this sheet has moved among the vagus rootlets and advanced almost to the emerging hypoglossal roots. In another (No. 22) the migrating cells cover the entire ventral surface of the medulla just in front of the cervical flexure having met, across the raphe, those moving down from the opposite side. In other words, there exists at this period a band of superficial undifferentiated cells uniting the roof attachment on both sides which is not unlike the early pontine bridge in fetus of 30 mm. The former begins just in front of the cervical flexure and subtends one-half to two-thirds of the olivary complex (fig. 2), but unlike the latter many cells leave it everywhere and make their way into the substance of the medulla to form gray matter in the interior.

It is striking (1) that the neuroblasts of the developing arcuate nuclei imitating the pontine formation, pay no attention to the raphe but cross it in an uninterrupted sheet; (2) that they appear before the anlage of the nuclei pontis, and if one turns to mammalian embryos (I have studied pig and rabbit) which are slightly larger than 20 mm. in crown-rump measurement, (3) that a well-developed arcuate formation exists just as in the human material.

Turning to the adult brain we find each arcuate nucleus a discrete mass which is separated from its counterpart by the raphe. Moreover the arcuate nucleus is peculiar to man so that from a phylogenetic standpoint we should expect to find it developing later than the pontine nuclei inasmuch as it is last to be acquired. Furthermore, very soon after its formation in pigs and rabbits one looks for it in vain. At 51 mm. only comparatively few cells can be found, while the superficial layer of migrating cells has disappeared completely from the subolivary region of a fetal pig of 60 mm. In man, on the other hand, when once there is a collection of cells over the medulla in the subolivary region (as in

No. 22) all of the later stages invariably show nuclear material in the position which we know will be occupied by arcuate nuclei. There appears but one rational explanation which will harmonize all of these apparently jarring facts which we have determined. In human embryos at the beginning of the second month there is an intramedullary migration of cells from the rhomboid lip to make up the olive, toward the end of the month the path of migration becomes more and more superficial until many of the cells actually cross the raphe before plunging into the medulla. In the lower mammals the comparatively simple olivary complex soon acquires its allotment of cells and when production of olivary neuroblasts ceases in the roof attachment, those on the surface soon find their way into the interior. In man, on the contrary, before the olive has received all of its cells and while the migration from the rhombic lip is still proceeding actively, neuroblasts which cannot be differentiated from those destined for the olive, begin to wander over the surface among the vagus roots. These elements stop on the ventral surface near the raphe and constitute the anlage of the arcuate nucleus. Stated differently, we are probably not dealing with arcuate formation in human embryos of 20 mm. where a cell lamina lies on the surface of the medulla in the place where we know the arcuate nucleus ought to be.

Just when the arcuate neuroblasts begin to descend from the rhombic lip can only be conjectured; this uncertainty has led me to call it 'olivo-arcuate migration.' Probably at 30 mm., as exemplified by No. 86, most of the thick superficial sheet of cells in the arcuate region represents a migration of olivary elements (fig. 10). Here the deeply staining nuclei form a continuous lamina over the ventral surface, the caudo-cephalic extent of which corresponds to the spinal one-half of the olive. Even older embryos present this pons-like structure as figs. 4, 5, and 6 illustrate. First in a fetus of 80 mm. (No. 172) does one meet with any large number of superficial neuroblasts under the cerebral one-half of the olive. Here almost the entire surface of the medulla is the seat of cellular migration. From the cervical flexure almost to the pontine nuclei the pyramidal tract is covered

by a superficial sheet of cells many of which are pushing their way into the medulla near the raphe. The wandering of cells to the region of the cerebral half of the olive fills up, in this embryo, the gap between the pons and the band (olivo arcuate migration, (fig. 2) which was first completed in embryos of 20 mm.

The addition of new elements is even more marked in the 96 mm. fetus (No. 484) where great numbers of moving cells are directed toward the ventral portion of the medulla immediately behind the pons. Here the cells are leaving not only the rhombic lip to pursue a course similar to the earliest olivo-arcuate migration but also from the ventral edge of the thick column of migrating pontine nuclei. All along the corpus ponto-bulbare of this fetus neuroblasts can be seen to leave its ventral edge and migrate directly toward the ventral median fissure. In the adult it is well known that the arcuate nuclei fuse across the midline as one nears the pons, although at the caudal end of the olive they present two discrete swellings which lie some distance from the midline. Two mechanical factors are concerned in breaking up this uninterrupted sheet of nuclear material which is so striking in the younger fetus (fig. 10). These are the formation of the external arcuate fibers and the growth of the pyramidal tract. Already in this fetus a considerable number of arcuate axones are crossing in the raphe, the main mass of nuclei, however, still lie on either side of the midline (fig. 11). It remains for the constant interstitial addition of pyramidal axones to bring about the further separation of the arcuate nuclei. It is apparent that the cortical projection system must occupy a very inconspicuous part of the cross sectional area of the medulla of this embryo, when one considers the superficial position of the olivary complex and their proximity to the ventral medial fissure. Compare with this the fig. 12 which is a camera lucida tracing of No. 508 (143 mm). The level of this section was made to correspond with that of No. 484 by choosing both about one-tenth of the distance from the caudal to the cephalic pole of the olive. The extensive addition to the pyramidal tract has pushed the olives apart as well as the arcuates. The latter, remaining superficial to the rapidly growing nerve system, have been drawn away from one another.

Along the raphe can be found a few cells, the remains of the connecting bridge, and these persist in this position even in the adult. Farther laterally one may often find small isolated masses at almost any point along the periphery of the medulla, the number

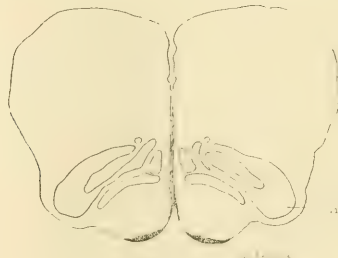
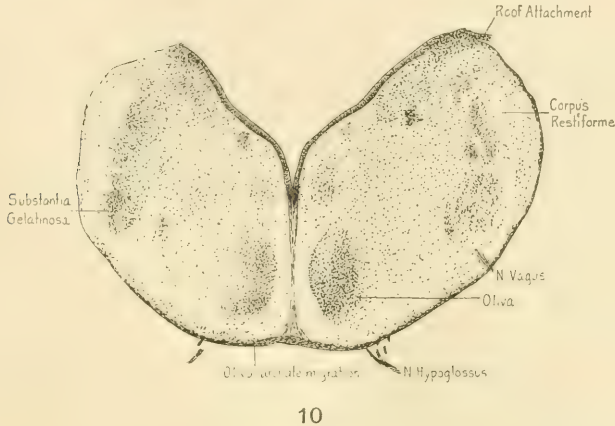


Fig. 10 Cross section through the lower olivary region of a 30 mm. fetus. $\times 16.5$. (No. 86, sl. 28, sect. 13).

Fig. 11 Camera lucida tracing of a cross section through the lower olivary region of a 96 mm. fetus. $\times 8.2$. (No. 484, sl. 3, row 2, sect. 7).

Fig. 12 Camera lucida tracing of a cross section through the lower olivary region of a 143 mm. fetus. $\times 7.3$. (No. 508, sl. 3, row 4, sect. 2).

and amount varying with different brains. These represent portions of the basilar nuclei which have not descended to the position of the arcuate nuclei proper. It will be remembered that in No. 508 many of the pontine nuclei are assuming ganglionic

form. This is not true in the arcuate formation where the nuclei are still very densely staining and there is very little protoplasm in the bodies. In No. 509 (188 mm.), however, the protoplasmic body is represented by a clear unstained area around a pale vesicular nucleus. Here it is possible to speak of young ganglion cells with certainty although most of the elements are still undifferentiated.

To conclude, then, we have in the rhombic lip or 'Rautenlippe' of His a common ancestor for the olive, pontine nuclei and arcuate nuclei—the nuclei pontis being formed by a migration through a restricted pathway, the corpus ponto-bulbare; the nuclei arcuati along with part of the olive by a superficial migration over the ventral surface of the medulla.

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THE SHEATH OF THE SINO-VENTRICULAR BUNDLE¹

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FIVE FIGURES

The present investigation, undertaken at the suggestion of Professor Meyer, is concerned chiefly with certain aspects of the connective tissue sheath around the atrio-ventricular bundle, or, as suggested by Retzer, the sino-ventricular bundle. As this sheath has recently been mentioned and described to a greater or less extent by many investigators, it is hoped that the present communication will be of some interest.

A review of the literature upon this subject will be given only so far as the connective tissue sheath is concerned. Kent in 1893, noted a considerable development in the region of the auriculo-ventricular groove. In 1906, Tawara while recognizing the Purkinje fibers as the branchings of the bundle, stated that the bundle and its branches were everywhere isolated from the cardiac muscle by connective tissue up to the point where the transition into muscle cells takes place, and that at this point also a transition of the connective tissue sheath into perimysium occurs. Keith and Flack in 1906, Fahr in 1908, and Mönckeberg in 1908, were impressed with the fibrous sheath and confirmed Tawara's findings. De Witt in 1909, while making dissections of the bundle and its branches preparatory to reconstructing it, noted that she could not remove the sheath because of the danger of breaking the strands of the bundle in many places along the finer branches and at the region of the nodal points.

¹It is believed that this is the first application of the injection method to the demonstration of the ramifications of the conductive system. These results are so easily obtained and give such a complete picture of the system, especially in the left ventricle, that after testing it the editor cannot refrain from thus calling attention to the value of the method.

Her models are, therefore, representations of the bundle and connective tissue sheath. Curran in 1909, stated that a mucous bursa, was constantly present in relation to the bundle and its branches in the human, calf, beef, and sheep hearts which he examined. According to Curran, the so-called bursa is best demonstrated by gross dissection, but he also succeeded in distending it by a blow pipe, the character of which was however not stated, and in many cases, saw bubbles of air in the region of his 'reticulum' upon the interauricular septum, and upon the sides of the interventricular septum, along the courses of the right and left branches. What Curran means by 'reticulum' is not quite clear, but presumably he refers to the gross structure of the bundle in the region where the 'Knoten' (Tawara) is found. He also stated that the bursa contains a fluid which is of greater consistency and more tenacious than ordinary lymph. In some cases, it was said to be so plentiful as to exude in the form of a droplet when the bursa was punctured, in others the overlying tissues could be made to puff out by making pressure on both ends of the branch. The place where this bursa was said to reach its greatest dimensions was just under the cartilaginous part of the septum, where the impact or friction with surrounding parts would presumably be greatest. From his microscopic work Curran concluded that the nature of this space varies from "very loose areolar tissue with fluid in the cellular spaces, which always connect with each other along the line of the bundle, to distinct cavities, filled or lubricated with fluid and no trabeculae crossing the intervening space. In the usual form, there are one or two large spaces or one continuous space with very fine trabeculae crossing from the walls of the canal to the auriculo-ventricular muscle." Curran, however, does not state whether these spaces are lined with a special endothelium or by a membrane similar to synovial membranes.

Dogiel in 1910, sought to throw doubt upon the existence of the auriculo-ventricular system as a muscular connecting link between the heart chambers and stated that the origin and the termination of such a *nerveless* bundle, its relation to the nerves, to the connective tissue, and to the rest of the heart muscle, are anatomically and physiologically unknown, and according to him, no

evidence has been shown to prove that the fasciculi of the bundle are separated from the heart muscle by connective tissue. Dogiel also states that the endocardium is not sufficiently transparent so that the fasciculi of the bundle and connective tissue sheath can be seen with the unaided eye!

The subject of the 'Sinus-knoten,' upon which Keith and Flack, Keith and Mackenzie, Fahr, Mönckeberg, Koch, Thorel and Wenkebach have done recent investigations, and the relations of the 'Sinus-knoten' to a connective tissue sheath, are not considered in this article, for the writer has sought to limit himself to the relations of the connective tissue sheath of the 'Knoten' and the parts of the bundle peripheral to this.

In the present investigation eighty-seven hearts were used; of these thirty-eight were beef hearts; thirty sheep, fifteen calf, and one was from a lamb six weeks old. About three-fourths of these hearts were used for injections, and many of them were afterwards dissected. Others were used in part for microscopic work. The rest were used for both gross dissection and microscopic work. Pieces of tissue including parts of the bundle with some of the cardiac muscle were removed from regions (where the bundle begins its course from the place) of the 'Knoten,' where the bundle passes under the cartilaginous septum and the main branches pass down upon the latter, and also farther down from regions where the branches of the bundle were very small. Serial sections of the complete system were not made, however, for it was thought that an examination of the bundle with its connective tissue envelope in different regions in a number of hearts would be entirely adequate for the purpose of this investigation. The tissues were fixed in formalin or Zenker's fluid, dehydrated, embedded in paraffin, and cut 5 to $7\frac{1}{2}$ and 10 micra in thickness. The ordinary haematoxylin and eosin stain was used, although some sections were stained with Van Gieson. In some instances where tissue was removed for microscopic study after injection of the sheath, the celloidin method was used.

Since a description of the atrio-ventricular bundle as it is seen by gross dissection would be a useless repetition of what has already been well done by others, it is omitted here. The bundle is easily located and easily dissected from its origin to the terminal

fasciculi and in all essential points I found it as described in the literature.

Although twenty uninjected hearts of beef, calf, and sheep, and others which were injected, were carefully dissected to determine the presence of a bursa, no evidence whatever of the existence of such a structure was obtained. The sheath and bundle with its larger and smaller branches could not be freed from its bed until the strand of connective tissue uniting it with the underlying musculature had been broken. Between the bundle and the heart muscle there seemed to be a line of cleavage in places where the connecting trabeculae were very fine and consequently broke easily, yet, these fine connections were always present. Under the cartilaginous or the bony septum of the beef heart, where the main bursa was located by Curran, a considerable amount of loose connective tissue was constantly present in the hearts which I examined, but a definite and preformed bursal space was never found. At the point where the left branch of the main bundle passes out from under a layer of muscle to lie immediately beneath the endocardium, a slight depression was noted usually in the endocardial layer. When the endocardium was carefully dissected off from this place and the edge of the layer of muscle lifted up with a forceps, very fine trabeculae were constantly seen connecting the muscle with the sheath of the bundle. Since this was the place where Curran located the main bursa, and where he stated the greatest friction existed between heart muscle and bundle we should expect some evidences of a bursa here. If, as Curran seems to think, a protective mechanism be necessary this loose connective tissue sheath could perhaps serve for protection of the bundle against the forcible impact of the surrounding structures.

A careful examination of the stained section from uninjected specimens failed likewise to reveal any space. On the contrary, the sections showed a thick layer of connective tissue enclosing the bundle. On the one hand, this layer passed over into the connective tissue of the cardiac muscle; on the other, it formed the envelope and framework within which the bundle lay embedded so that each strand had its sheath. The stained sections further

showed that the attachment of the sheath to the individual fasciculi was relatively loose, and whether the latter were regular or irregular, the sheath was usually closely applied to them, although a slight separation was seen in some cases. In the main mass of the bundle, small blood vessels, nerve bundles and some fat were noticed.

Repeated attempts to demonstrate the presence of a fluid as found by Curran, were made by pressing on the superficial parts of the bundle. For this purpose, hearts were chosen where the main branches of the bundle were superficial enough to form a plainly visible and palpable strand under the endocardium. Since the right branch of the bundle is more cylindrical in form and lies upon the convex side of the septum, it seemed better adapted for this purpose. If a bursa containing fluid exists, it should be found most easily along this part of the bundle. However, when pressure was made at distant points and the fingers were made to approach each other, no bulging or pouching of the endocardium from fluid pressure underneath occurred. What happened was a gathering of the endocardium and the tissues superficial to the bundle into a number of ridges or undulations, running transversely to the long axis of the bundle. These results certainly speak against the existence of fluid in a bursal space and can have resulted only from the tissues being bound down tightly to the underlying structures. Attempts to withdraw fluid by means of a very fine capillary pipette were also unsuccessful.

The injection of various fluids into the tissues directly beneath the endocardium surrounding the bundle and its branches, was also used as a means of demonstrating the presence or the absence of a bursa. For this purpose sixty hearts were used. Twenty-nine of these were beef hearts, twenty-four sheep, six calf and one from a six weeks lamb. An ordinary hypodermic syringe with a relatively fine needle was used for these injections. Watery suspensions of India ink, of finely precipitated Prussian-blue, of Prussian-blue gelatine, and at times air, were used as injection media. These were injected into the tissues between the bundle and the endocardium, and between the bundle and the heart

muscle along both the right and the left branches. At times the needle was pointed downward, at others, upward. In order to reach the point just under the cartilage in the ox heart where Curran described a bursa a centimeter in diameter, the needle was inserted from high up on the left side of the septum under the right cusp of the aortic valve, and directed downward and to the right a distance of 8 to 10 mm., this procedure being employed as a result of examination of dissected specimens. The amount of pressure used in making the injections was not gauged, but it was very slight, firm pressure never seeming necessary.

The results of the first injections were good pictures of extravasation, and subsequent dissections showed this to be the case. No confining limits could be made out, for the injection material extended as readily into the connective tissues between the cardiac muscle fibers and out under the endocardium as into the tissues immediately around the bundle. This extravasation was found in all regions, whether along the course of the main bundle under the cartilaginous septum, or along the larger or the smaller branches of the system. Hence, unless the walls of a preformed space had been ruptured by pressure, which was unlikely, we must conclude that a bursa, if it exists, must extend out under the endocardium and in between the cardiac musculature. Microscopic examination of stained sections also showed the diffuse character of the injection mass typical of extravasation. In the case of beef heart no. 12, however, a different result was apparently obtained. In this specimen, the attempt had been made to inject the 'main bursa,' but accidentally the sheath of the bundle had been pierced and the injection solution delivered within it. On dissecting the main bundle from the 'Knoten' to the division into the septal branches, it was seen that, the injection mass was confined entirely within the sheath except at the point of puncture, where some fluid had extravasated. This result suggested the idea that it might be possible constantly to inject the sheath, and by using air in the syringe it was also found that the sheath could be inflated. If, for example, air was injected from the right side into the sheath of the right branch at the base of the moderator band or trabecula supraventricularis, it ran up

towards the main branch and, in some instances, at once appeared on the left side within the sheath of the left branch. Following out this same idea, a complete injection of the system was obtained from its point of departure from Tawara's 'Knoten' to the very fine ramifying branches which lie under the endocardium, and even to the finest terminal fasciculi which end in the myocardium. The chief difficulty was to insert the needle at just the proper depth to pierce the sheath. The point selected for the purpose of injection was immaterial as long as the fasciculus chosen was somewhat thicker than the needle.

Contrary to Dogiel's view, the endocardium is always transparent enough to permit accurate localization of the fasciculi. These are readily seen as they pass along under the endocardium, and after some trials few mistakes were made in inserting the needle.

Twenty-five hearts were used for the injection of the sheath in this manner, eighteen of these were fresh beef hearts, and one a lamb's heart, the rest being formalin-preserved hearts of cattle. In all of these a practically uniform result was obtained. The sheath of the atrio-ventricular bundle could be filled with the colored fluids without difficulty. The lamb's heart gave an especially delicate and fine picture of the injection. However, as a rule, it was difficult to force the fluid upward to the main bundle and farther upward to the region of the 'Knoten,' although this was done in some cases.

Fig. 1 is a photograph of beef heart no. 36 of this series, and although the figure is a retouched photograph it does not represent adequately the extent nor the completeness of the injection. This heart was opened by an incision which extended up from the apex parallel to, and about three centimeters from the anterior longitudinal sulcus. The view shows the left ventricle with its outer wall reflected to the left. The injection mass used was a suspension of India-ink. Points of injection were at 1, where the left branch appears superficially and begins its downward course, and at 2, where some of the ramifying divisions of the left branch have come to lie upon the posterior papillary muscle. The fluid, injected at 1, ran down within the sheath of

the left branch to the region where this divides at about the junction of the upper and middle thirds of the septum. From here, three main paths lead off; an anterior which traversed a false tendon to reach a papillary muscle, a middle which passed down to branch out upon the septal wall as far as the apex, and a posterior which crossed a false tendon to reach the base of the pos-

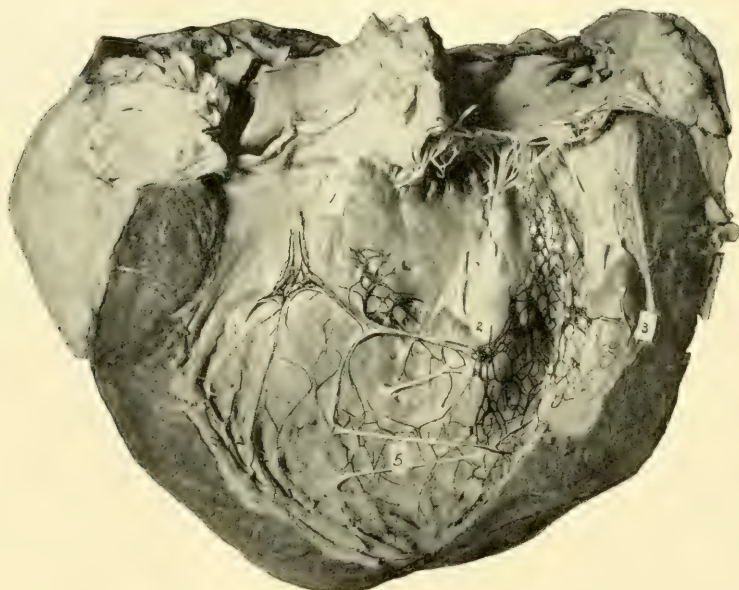


Fig. 1 Photograph of ox heart, retouched, showing injected sheath of atrio-ventricular bundle. One-half actual size. 1 and 2, points from which entire left side of sheath was injected; 3 and 4, a cut false-tendon, connecting the septal with the outer wall of the chamber; 5, marks the region above apex, where a very fine anastomosis is not well represented in the photograph.

terior papillary muscle. On the septal wall, the whole sheath was injected from the one point 1. In the same way the system of branches on the outer wall of the ventricle was filled from point 2. The false tendon connecting points 3 and 4 was afterward cut to facilitate photographing the specimen. At 5 two tendon threads are shown which carry Purkinje fasciculi, but the sheaths of these were not completely injected.

A detailed description of the coursing of the finer branches as shown in fig. 1 is unnecessary, but a short summary seems justified. On the septal wall the fibers unite, branch and reunite to form a network, the meshes of which constantly grow smaller until in the apex of the heart a mantle of fine anastomosing fibers occurs. This lies at a region under δ and is not well shown in the figure. Upon the outer wall of the ventricle, the injected meshwork extends from the apex almost to the attachment of the leaflet of the mitral valve. Here, the fasciculi appear relatively smaller, unite at more frequent intervals than those upon the septal wall, and form a finer network. Moreover, the points of union are more characteristically node-like. Often, fine fasciculi, unnoticed before injection, became apparent and could be seen to branch and to unite with others until they were finally lost to view because the sheath was uninjected.

With the sheath system in the right ventricle similar results were obtained. The fluid ran down in the sheath of the right branch and passed to the outer wall of the chamber by way of the moderator band, where anastomoses occurred between the branches, as in case of the left ventricle. From the outer wall other strands travelled back to form a septal network. The right division, however, appeared somewhat harder to inject than the other, possibly because of a closer attachment of the sheath. Upwards, at the beginning of the interventricular septum, it was found that the injection had not extended to include the main branch. This, also, may have resulted from a too firm union between sheath and bundle, or perhaps from the use of too little pressure. Dissection of beef heart no. 37, the next in the series, showed that the sheath of the main bundle had been filled with fluid as far as the interauricular region—the 'Knoten'—and other specimens showed the same condition.

In the heart of the young lamb only the left side of the system was injected. Here, too, a very fine and delicate system of anastomosing fasciculi, similar in all respects to those in the larger beef hearts, was brought into view. With the formalin-preserved hearts of beef and calf, a like result was obtained but the extent to which the fluid would penetrate within the sheaths of these was

naturally limited because of the hardening and loss of elasticity of the tissues.

That the sheath does not simulate a bursa in containing fluid was well shown by injecting a fasciculus from two points, when the fluids would run together perfectly without hindrance from any other contained fluid. Attempts to demonstrate a lining by means of a silver stain were also unsuccessful nor was it possible to demonstrate microscopically the existence of definitely arranged nucleii which the connective tissue cells of the inner layer of a bursa should show.

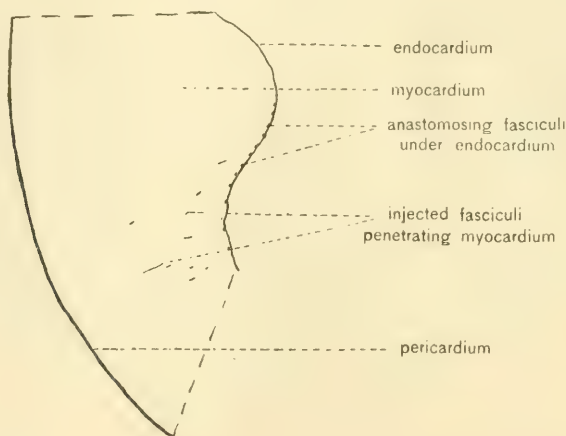


Fig. 2 Ox heart; cross-section; diagram of left ventricular wall, actual size, showing depths within myocardium at which the injected branches of the sino-ventricular system could be traced with the naked eye.

Tawara, who traced the Purkinje fasciculi and their sheaths through the myocardium to their terminations or transitions into heart muscle and perimysium respectively, states that the sheaths everywhere form a closed system and isolate completely the fasciculi from the heart muscle. From an examination of my specimens these statements are wholly confirmed, for even with the naked eye, it was possible to find injection masses in some instances as far as fifteen millimeters in the myocardium. Fig. 2 is a diagram of part of the left ventricular wall of beef heart no. 26, and illustrates this fact. Furthermore, it was possible, by

securing serial sections of pieces of myocardium containing parts of the injected system, to trace on the one hand, the sheath with the enclosed injection mass as far upward in the region of the 'Knoten' as the connective tissue of the auricular muscle, and on the other as far into the ventricular wall as the point of termination of the Purkinje fibers, where the sheath becomes the perimysium.

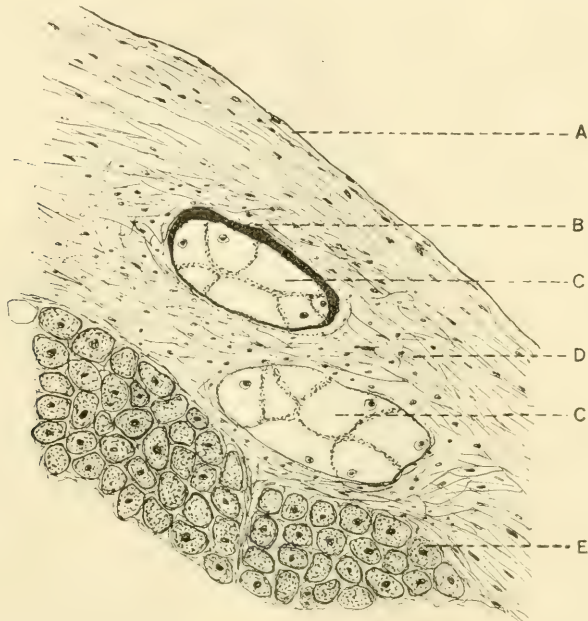


Fig. 3 Section showing two Purkinje fasciculi under the endocardium. *A*, endocardium; *B*, injection mass within connective tissue sheath; *C*, Purkinje fasciculus; *D*, connective tissues; *E*, cardiac muscle. $\times 275$.

For this microscopical work, pieces of tissue including parts of the injected system were taken from the different regions, i.e., from the region of the 'Knoten,' from that of the main bundle, of the main branches, from the sub-endocardial anastomosis, and from the terminations within the myocardium. Paraffin and celloidin were employed for embedding and the sections were stained with haematoxylin-eosin and van Gieson. Where the fasciculus had appeared as a definite strand in the gross, the micro-

scopic examination showed that the injection mass was entirely held within the connective tissue envelope. At points, this sheath had been dissected and separated around the entire circumference of the fasciculus. At other points the attachments of the sheath were still partly intact and limited the injection material to a part of the circumference. Where the strands anastomose, or where a side branch is given off, as Tawara has stated, the connective tissue follows each part, giving it a complete investment. This was well shown by studying serial sections of a part of the bundle where numbers of strands go to form one large branch. The India-ink, or the Prussian-blue mass made the tracing of the sheath easy, for by looking backward and forward in the series the anastomosis of the near-by fasciculi enveloped by a continuous connective tissue covering was marked by the continuity of the enclosed black or blue mass.

Fig. 4 shows a camera lucida drawing of a section of tissue from beef heart no. 26, the section being cut somewhat obliquely to the long axis of the cardiac muscle cells. The figure shows the transition point of a Purkinje fiber into the heart muscle, the fiber showing some of the India-ink injection mass within its sheath. In the series from which this section was taken the fiber *A* is found to be a branch from *B*, the sheaths and the enclosed injection material of the two strands, therefore, are continuous. The fasciculus *B*, traced through the cardiac tissue for a relatively great distance, gives off the short fiber *A* which passes at once to the wedge-shaped fasciculus of cardiac muscle cells *E*, and becomes lost in the latter at the apex. As shown in the figure, the injection material had travelled along in the sheath-like covering of the fiber *A* to the point where this change takes place and the sheath is seen to be directly continuous with the perimysium of the heart muscle.

This mode of transition of a fiber into a group of muscle cells, is apparently one of the ways in which the sino-ventricular system may terminate within the myocardium of the ventricles. Tawara has discussed other forms. While an inquiry into these terminal mechanisms and the histology of the same, has not been the special object of this investigation, nevertheless, in tracing out the ramifications of injections, into the sheath, the above

transition was observed. Such a transition could, perhaps, be considered a means to offset the great numerical disproportion which must exist between the fibers of the atrio-ventricular system and the heart muscle fibers.

Fig. 5 shows a camera lucida drawing from the auricular region—the 'Knoten'—in a section of tissue from beef heart no. 12.

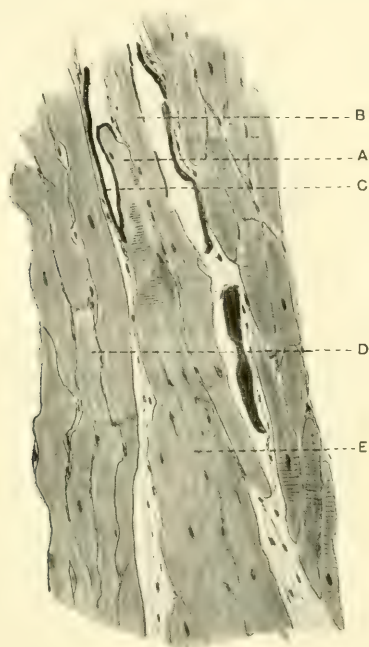


Fig. 4 Camera lucida drawing; section of ventricular myocardium, beef heart. *A* and *B*, Purkinje fasciculi; *C*, injection material within connective tissue sheath; *D*, cardiac muscle fibers; *E*, fasciculus of heart muscle fibers in which fiber *A* terminates. $\times 275$.

Point *A* shows the auricular muscle fibers cut in cross section. From these, bundles of fibrillae pass off to enter a point of union for many such fibril fasciculi, which place Tawara has called a node. In this nodal point the fibrils are seen as confused strands coursing in many different directions. The clear spaces *G* represent the indentations of the node which have been cut in the sectioning, and within which the connective tissue nuclei can be

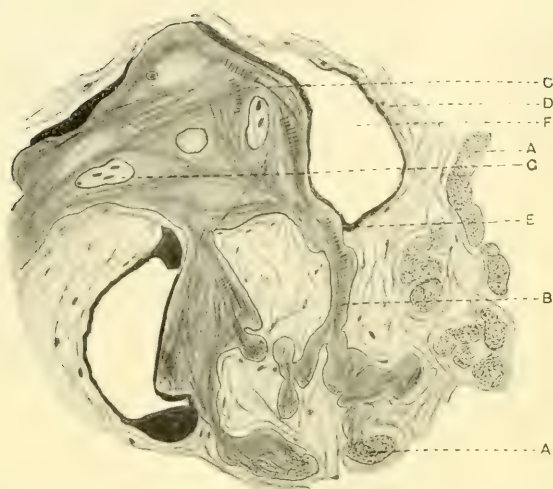


Fig. 5 Camera lucida drawing; section from region of the 'Knoten,' showing a large nodal point with connections. *A*, cardiac muscle fibers (auricular); *B*, fibril strands from muscle fibers to the node; *C*, cross striations of fibril bundles within the node; *D*, connective tissue; *E*, injection mass between connective tissue and node; *F*, spaces produced by injection mass; *G*, indentation in node, showing connective tissue within.

seen. Cross striations of the fibril strands are plainly seen at many points *C*. Surrounding these structures is the connective tissue *D* which forms the sheath of the atrio-ventricular system in this region, and which encloses the injection mass *E* within it. Since uninjected specimens, do not show such a great separation between sheath and system, it seems probable that the large spaces *F* were made by the injection and later fixation and dehydration of the tissues kept them in the distended position while the injection material settled to a small mass.

There exists, then, in beef, calf and sheep hearts, a complete connective tissue envelope applied to the sino-ventricular system in all its ramifications. It clothes the system in its entirety beginning above at the point where the main bundle takes its origin and continues downward along the branching ventricular fasciculi to their terminations. In the sub-endocardial region, it is in relation with the sub-endocardial connective tissue, while within the myocardium it is related to the interstitial myo-

cardial connective tissue. It is remarkable that the sheath can be so easily dissected loose from the bundle which it covers and that it can nevertheless withstand the pressure necessary to inject it so extensively. Although a rapid diminution of pressure undoubtedly occurs as the fasciculi branch and anastomose, the pressure at the point of injection is relatively great; nevertheless the sheath withstands it, if ordinary care be used. Furthermore, in view of the reduction of pressure which must result from the progressive division and anastomosis of the fasciculi the extent to which the sheath can be injected is very great.

The question might well be asked whether or not this system, which can be injected so easily, is in relation with the lymphatic system. A consideration of the origin and the termination of the sheath will furnish the answer. It has been shown that the tissue forming the sheath is continuous, on the one hand, with the perimysium of the auricular muscle, on the other with the perimysium of the ventricular fibers. Consequently, if we look upon the sheath as composing the walls of a lymph space we must necessarily look upon the perimysium in a similar way, and accept the fact that this lymph space extends out around the fasciculus of heart muscle cells and around the single cells. Such a conception would of course seem untenable.

The functions of the sheath lie outside the scope of this paper, excepting so far as the idea of a lubricating mechanism is concerned. If, by a bursa, we understand a structure distinct from the sheath, the existence of such a bursal space was not confirmed by this investigation. On the other hand, if the inflated connective tissue sheath was mistaken for a bursa, the interpretation of its function as a lubricating apparatus seems impossible. Other writers, notably Tawara, Keith, Fahr, and Mönckeberg, have spoken of its function as one of isolation and insulation. However, Dogiel, whose opinion is worthy of great consideration, has recently stated that the existence of such a sheath has never been satisfactorily proven, and hence feels under no necessity to ascribe to it a function. The writer offers the facts herein reported as further proof of the indisputable existence of the sheath as a very definite structure which isolates the atrio-ventricular system from the rest of the heart, even to its terminations.

CONCLUSIONS

1. In hearts of beef, calf and sheep there is a definite connective tissue sheath surrounding the sino-ventricular bundle, which completely invests and isolates that bundle from the heart muscle.
2. The existence of this sheath can be demonstrated in its entirety by injections, for the sheath is capable of maintaining its integrity against considerable pressure from within.
3. This sheath begins above where the fasciculi of the bundle are continuous with the musculature of the interauricular septum (Tawara's 'Knoten') and clothes the system up to its ventricular termination, i.e., to its transition into cardiac muscle fibers.
4. The sheath does not simulate a bursa, save very remotely, perhaps, nor is it part of the lymphatic system of the heart.
5. Hence this sheath is not a bursa and no synovial bursa exists, for if a bursa be present in any portion, these injections show that it is coextensive with the bundle itself.

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THE DEVELOPMENT OF THE ADRENALS IN THE TURTLE

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NINE FIGURES

CONTENTS

Introduction.....	71
Observations.....	76
Early development.....	76
Cortical substance.....	76
Chromaffin substance.....	79
Later development.....	81
Genetic relationship and differentiation of chromaffin cells.....	85
Summary.....	87
Bibliography.....	88

INTRODUCTION

A review of the literature bearing on the development of the adrenals in the several classes of vertebrates reveals several opposing theories of development each of which has been supported by investigators of recognized ability. The adrenal system is a compound system. In the lower vertebrates it includes (according to the nomenclature proposed by Balfour) the interrenals and the suprarenals. In the higher vertebrates these two systems are consolidated more or less completely into a single pair of adrenal organs composed of the cortical substance which corresponds to the interrenals of the lower vertebrates, and the medullary, or chromaffin,¹ substance which corresponds to the suprarenals. These organs have been described by some investigators

¹ The term, chromaffin substance, will be used in this paper in preference to the term, medullary substance. A true medulla occurs only in the adrenals of mammals.

as having a homogeneous origin; i.e., both the cortical and the chromaffin substance arising from the same source, and by others as having a heterogeneous origin; i.e., the cortical and the chromaffin substance arising from separate sources. The advocates of the theory of the homogeneous origin of the adrenals have in turn derived them from the mesenchyme, the peritoneal epithelium, the germinal epithelium, the epithelium of the pronephros, the epithelium of the mesonephros and from the peripheral part of the sympathetic nervous system. The advocates of the theory of the heterogeneous origin of the adrenals have in turn derived the cortical substance from the mesenchyme, the peritoneal epithelium, the epithelium of the pronephros and the epithelium of the mesonephros, while they have in turn derived the chromaffin substance from the mesenchyme and from the sympathetic nervous system.

A comprehensive review of the literature bearing on the development of the adrenals would be foreign to the purpose of this paper. An attempt will be made only to indicate the trend of the progress of our knowledge in this field by brief reference to some of the more important papers. For a more comprehensive review of the literature and for a complete bibliography, the reader is referred to the work of Poll² ('06).

The most important investigations on the adrenal system before the classical work of Balfour are those of Leydig ('53). This investigator described both the interrenals and the suprarenals in the fishes and concluded that the latter are derived from the sympathetic nervous system. Indeed, he says, "As the pituitary body is an integral part of the brain, so are the suprarenal bodies part of the sympathetic system."

The work of Balfour ('78) shows conclusively that in the elasmobranch fishes the interrenals are of mesodermal origin, while the suprarenals are derived from the sympathetic ganglia located along the abdominal aorta. The findings of Balfour were corroborated by the work of some of his pupils, notably that of Mitsukuri ('82).

² Hertwig's Handbuch, 1906, vol. 3, part 1, chap. 2, pp. 443-616.

Although the theory that the suprarenals, or the chromaffin substance, are derived from the sympathetic nervous system was so well supported by the work of these early investigators, later observers have repeatedly failed to recognize the nervous origin of these bodies and have attempted to derive them from some other source. This condition is probably due in part to a failure to recognize the homology of the chromaffin substance of the adrenals of the higher vertebrates with the suprarenals of the fishes and in part to an erroneous interpretation of observations coupled with an unwillingness to believe that any part of the adrenals is derived from the sympathetic nervous system.

Gottschau ('83), working with mammalian embryos, noted the intimate association of the chromaffin substance with the cortical substance and concluded that both arise from the mesenchyme. Janosik ('83) observed that in mammalian embryos cells advance from the peritoneal epithelium into the mesenchyme in the region in which the adrenals arise. He concluded, therefore, that these bodies are derived from the germinal epithelium. Like Gottschau he believed that the cortical and the chromaffin substance are derived from the same source. O Schultze ('97) concluded, from observations made on embryos of *Vespertillio murinus*, that the entire adrenal anlage is derived from sympathetic ganglia and that it is later differentiated into a cortical and a medullary portion. Minot ('97) found no evidence of the sympathetic origin of any part of the adrenals in human embryos, but supported the older view of Gottschau that both the cortical and the chromaffin substance are derived from the mesenchyme.

The above citations are sufficient to indicate that there has been no general agreement in the conclusions to which the earlier investigators were led. A similar lack of agreement, though less marked, is also prevalent among the later investigators.

Flint ('00), in his paper on the blood vessels of the adrenals, does not attempt to determine the ultimate source of either the cortical or the chromaffin substance. He shows, however, that in embryos of the pig the cortical substance arises first and that the chromaffin substance arises from cells which wander in from the outside. That the cells which give rise to the chromaffin

substance are derived from the sympathetic nervous system, in his opinion, requires further proof.

Aichel ('00) advanced a new theory of the origin of the adrenals. According to this author, the interrenals alone in the selachians are homologous with the adrenals of the higher vertebrates. These, he believes, arise from the peritoneal invaginations of the pronephros. The so-called suprarenals in the selachians, he concludes, are derived from retrograding canals of the same body. In embryos of both the rabbit and the mole, according to Aichel, both the cortical and the chromaffin substance are derived from the epithelium of the pronephros. This view finds no support in the work of other investigators.

Wiesel ('01), although his observations were made on embryos of the pig which were too far advanced to reveal the earliest traces of the adrenal anlagen, concluded that the cortical substance is derived from the peritoneal epithelium, while the chromaffin substance is derived from the prevertebral sympathetic plexuses.

Whitehead ('03) studied the development of the adrenals in embryos of the pig from their earliest anlagen. His conclusions agree in general with the conclusions of Wiesel above cited.

By an exhaustive review of the literature and by extensive observations, Poll ('06) has shown that the weight of evidence is in favor of the view that in all the classes of vertebrates the cortical substance of the adrenals arises from the peritoneal epithelium, while the chromaffin substance is derived from cells which become separated from the anlagen of the sympathetic nervous system. The genetic relationships of the cells which give rise to the chromaffin substance, however, as well as the processes by which these cells become associated with and approximated to the cortical substance and by which they become transformed into typical chromaffin cells have not been understood.

During my studies of the development of the sympathetic nervous system in embryos of the Loggerhead turtle (*Thalassochelys caretta*), my attention was attracted by the phenomena involved in the development of the adrenals. Embryos of this species afford excellent material for the study of these phenomena because the chromaffin material is comparatively abundant, the

embryos are comparatively large, develop comparatively slowly and afford excellent histological preparations.

The development of the adrenals has been investigated less extensively in the Reptilia than in the other classes of vertebrates. Our limited knowledge of the development of these organs in this class of vertebrates is the more appreciable because the adrenals in this, the most primitive class of the Amniota represent a transition stage in the evolution of the highly specialized adrenal organs in the higher vertebrates from the more primitive adrenal system in the Anamnia.

Observations which have been recorded on the development of the adrenals in the Crocodilia and the Ophidia are only fragmentary and inconclusive. More or less extensive observations on the development of these organs in the Sauria, primarily in certain species of the genus *Lacerta*, have been recorded by Braun ('79, '82), von Mihalcovics ('85), Weldon ('85), Hoffmann ('89) and Soulie ('03). von Mihalcovics, being an advocate of the theory of the homogeneous origin of the adrenals, derived both the cortical and the chromaffin substance from the germinal epithelium. The other investigators above mentioned agree in deriving the chromaffin substance from the anlagen of the prevertebral sympathetic plexuses. They do not agree, however, as to the origin of the cortical substance. According to Braun, the cortical substance arises directly from the mesenchyme. According to Weldon and Hoffmann, it arises from the epithelium of the pronephros. According to Soulie, it arises from the peritoneal epithelium.

The only extended observations on the development of the adrenals in the Chelonia which have been recorded, as far as the writer is aware, are those of Poll ('04, '06) on embryos of *Emys europaea*. This author describes the origin of the cortical substance from the peritoneal epithelium in detail. He has little to say, however, concerning the chromaffin substance except that it is derived from the anlagen of the prevertebral sympathetic plexuses.

The following observations are based almost exclusively on embryos of the Loggerhead turtle (*Thalassochelys caretta*).

I take pleasure in expressing my indebtedness to Professor F. A. Stromsten for the use of a large number of embryos of this species which were collected by him at the Dry Tortugas, Florida, during the summer of 1910. It is a real pleasure also to express my deep sense of obligation to Professor G. L. Houser for helpful suggestions during the progress of this investigation and for reading the manuscript.

OBSERVATIONS

Early development

Cortical substance. The anlagen of the adrenals arise in embryos of *Thalassochelys caretta* during the eighth day of incubation as buds of cells which proliferate from the peritoneal epithelium just laterad to the root of the mesentery and approximately at the middle level of the mesonephros (fig. 1, *ad*). At the close of the eighth day of incubation a short series of buds may be observed on either side of the aorta rising into the mesenchyme between the aorta and the mesonephros. These buds are at first more or less wedge-shaped with a broad base resting on the peritoneal epithelium from which they arise (fig. 1, *ad*). There is no well marked differentiation apparent at this stage between the cells composing the adrenal buds and the cells of the adjacent mesenchyme. The nuclei of the former often appear somewhat clearer than the nuclei of the latter and their cytoplasm stains somewhat more intensely. These buds may be readily recognized, however, by the relatively compact and more or less regular arrangement of the cells composing them. The exact number of buds taking part in the development of the cortical substance of each adrenal organ is not easily determined. These buds do not all arise simultaneously. During the ninth and the tenth day of incubation, after the earliest buds have become nearly or completely separated from the peritoneal epithelium, buds may still be observed in the anterior region of the zone of proliferation which exhibit the earliest phases of their development from the peritoneal epithelium.

At the close of the eighth day of incubation, the buds in the middle region of the adrenal zone rise in the mesenchyme as high as the middle level of the aorta and in sections occupy the greater part of the area between the aorta and the mesonephros (fig. 2, *ad*). The cells in these buds are now arranged in more or less regular rows rising from the base of the wedge-shaped aggregate toward its apex which is usually slightly recurved toward the mesial surface of the mesonephros. Mitotic figures may occasionally be observed in these growing buds. It is obvious, therefore, that these cells retain the power of further propagation by division after they have advanced into the adrenal anlagen, thus giving the latter the capacity of independent growth after they are no longer connected with the peritoneal epithelium.

As development advances, the adrenal buds become larger and advance farther into the mesenchyme, rarely, however, rising but slightly higher than the middle level of the aorta. They no longer appear wedge-shaped in transverse sections, but have become rounded or oval in outline, retaining connection with the peritoneal epithelium only by a slender stalk (fig. 3, *ad*). During the tenth day of incubation, some of the buds become almost or completely separated from the peritoneal epithelium. They now appear in transverse sections as condensations in the mesenchyme between the aorta and the mesonephros and are often so closely approximated to the mesial surface of the latter that except for a slight difference in the staining properties of the cells composing them it becomes difficult to distinguish between these two complexes. Such conditions, doubtless, are responsible for the conclusions of some of the earlier investigators that the cortical substance of the adrenals is derived either directly from the mesenchyme or from the epithelium of the mesonephros.

At the close of the eleventh day of incubation, the adrenal anlagen have become separated from the peritoneal epithelium throughout nearly the entire extent of the adrenal zone. In transverse sections, they appear more or less circular in outline, being modified in form somewhat by the limitations of the area between the aorta and the mesonephros (fig. 4, *ad*). In the anterior and the middle region of the adrenal zone, the adrenal anlagen

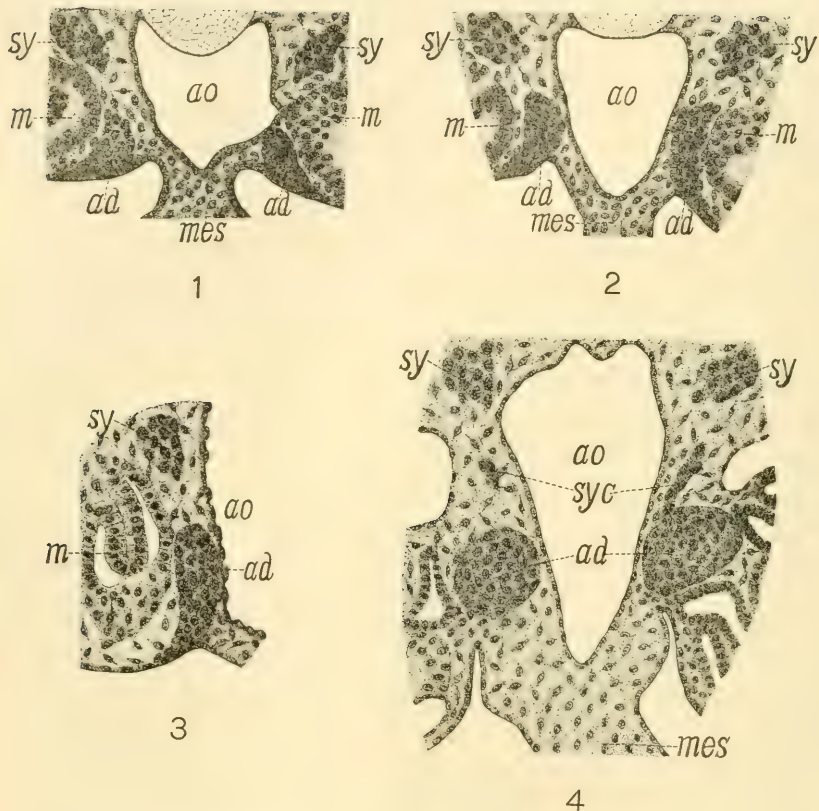


Fig. 1 Transverse section through the anterior region of the adrenal zone in an eight-day embryo of *Thalassochelys caretta*. $\times 150$. *ad*, adrenal buds; *ao*, aorta; *m*, mesonephros; *mes*, mesentery; *sy*, anlagen of sympathetic trunks.

Fig. 2 Transverse section through the middle region of the adrenal zone in an eight-day embryo of *Thalassochelys caretta*. $\times 150$. *ad*, adrenal buds; *ao*, aorta; *m*, mesonephros; *mes*, mesentery; *sy*, anlagen of sympathetic trunks.

Fig. 3 Transverse section through the middle region of the adrenal zone in a nine-day embryo of *Thalassochelys caretta*. $\times 150$. *ad*, adrenal buds; *ao*, aorta; *m*, mesentery; *sy*, anlage of sympathetic trunk.

Fig. 4 Transverse section through the adrenal zone in an eleven-day embryo of *Thalassochelys caretta*. $\times 150$. *ad*, adrenal anlagen; *ao*, aorta; *mes*, mesentery; *sy*, anlagen of sympathetic trunks; *syc*, sympathetic cells.

are now located below the middle level of the aorta, while in the posterior region they lie close to the ventro-lateral aspects of the latter and are not far removed from the peritoneal epithelium in the angle between the mesentery and the mesonephros. The primary arrangement of the cells in irregular rows is no longer apparent. At this stage, however, the cells seem to be arranged in more or less regular concentric layers.

At the close of the thirteenth day of incubation, the aorta has become relatively smaller than in the preceding stages and the internal organs are located farther ventrally with respect to the former. In the anterior region the adrenal anlagen now lie along the ventro-lateral aspects of the aorta, while farther posteriorly they lie distinctly below its ventral level (fig. 5, *ad*). In the posterior region the anlagen of the adrenals have become somewhat compressed dorso-ventrally and appear more or less oval in transverse sections. The mesial surfaces of the right and the left adrenal have now become closely approximated to each other in this region. Denser aggregates of cells may now be observed scattered irregularly throughout the section of the adrenal anlage. This condition, doubtless, represents the initial stage in the differentiation leading to the characteristic arrangement of the cells in the cortical substance of the adrenal gland.

After the close of the thirteenth day of incubation, the adrenal anlagen become somewhat farther removed from the aorta. They increase in size materially and the cells assume a more definite arrangement. At the close of the thirteenth day, the denser aggregates of cells noted above have become more conspicuous and are variously connected with each other by strands of closely aggregated cells (fig. 6, *ad*). The meshes within this complex stain less intensely than the denser areas and contain relatively few cells.

The observations thus far recorded agree in all essentials with the observations of Poll ('04, '06) on the development of the cortical substance of the adrenals in *Emys europaea*.

Chromaffin substance. The above observations pertain only to those parts of the adrenal anlagen which arise from the peritoneal epithelium and give rise to what is commonly known as

the cortical substance. The chromaffin substance arises in an entirely different manner. As has been repeatedly maintained by certain investigators and denied by others, for the several classes of vertebrates, and as has been shown by Poll also for the turtle (*Emys europaea*), this part of the adrenal system is derived from the anlagen of the prevertebral sympathetic plexuses. It is, therefore, primarily of ectodermal origin.

During the eighth day of incubation, when the adrenal buds appear as small cell-aggregates arising from the peritoneal epithelium, the anlagen of the ganglia of the sympathetic trunks are already present as irregular cell-groups lying in the mesenchyme approximately at the dorsal level of the aorta (fig. 1, *sy*). As development advances, the anlagen of the sympathetic trunks become larger and, as the writer has shown in an earlier paper ('11 a), cells become separated from them and migrating ventrally give rise to the anlagen of the prevertebral sympathetic plexuses which are located along the ventro-lateral aspects of the aorta.

During the eleventh day of incubation, sympathetic elements may be traced ventrally from the anlagen of the sympathetic trunks along the lateral surfaces of the aorta. In the anterior and the middle region of the adrenal zone where the adrenal anlage is still located between the mesonephros and the aorta, sympathetic cells may in some sections be observed in contact with the anlage of the cortical substance, but no such elements could be observed, at this stage, among the cortical cells (fig. 4, *sy c*).

At the close of the thirteenth day of incubation, sympathetic cells may be observed as far ventrally as the ventral level of the aorta and the anlagen of the prevertebral sympathetic plexuses have become well established. The adrenal anlagen now lie in close proximity with the anlagen of the prevertebral sympathetic plexuses and in some sections small groups of sympathetic cells may be observed in contact with the cortical mass (fig. 5).

As development advances, the cells composing the anlagen of the prevertebral sympathetic plexuses become more numerous until at the close of the nineteenth day of incubation the aorta is completely encircled ventrally by conspicuous aggregates of sympathetic cells (fig. 6, *pv*). The adrenal anlagen are now somewhat

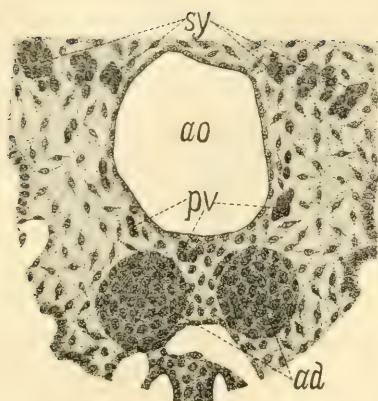
farther removed from the aorta and numerous small cell-groups may be traced from the larger cell-aggregates constituting the anlagen of the prevertebral sympathetic plexuses toward the adrenal anlagen (fig. 6, *cc*). Many of these cell-groups may be observed closely approximated to the dorsal and the mesial surfaces of the adrenal anlagen, while not infrequently such cells may be observed just beneath the surface of the adrenal anlage among the cortical cells (fig. 6, *cc'*).

There is no observable difference at this stage between the cells of sympathetic origin which advance toward the anlagen of the adrenals and the cells which remain within the sympathetic plexuses. That these are the cells, however, which give rise to the chromaffin substance associated with the cortical substance in the adrenal organs can not be doubted. These cells not only become aggregated at the dorsal and the mesial surfaces of the adrenal anlagen, but, as development advances, groups of these cells may be found completely encircling the cortical substance of the adrenals and penetrating into the spaces which appear between the aggregates of the cortical cells as the latter approach more and more closely the arrangement which is typical of the adrenal glands in the mature state. Furthermore, sympathetic cells do not advance farther ventrally into the mesentery at this level, and, as the writer has shown in the earlier paper referred to above, the cells which give rise to the sympathetic plexuses in the walls of the digestive tube are derived from other sources; viz., the hind-brain and the vagus ganglia.

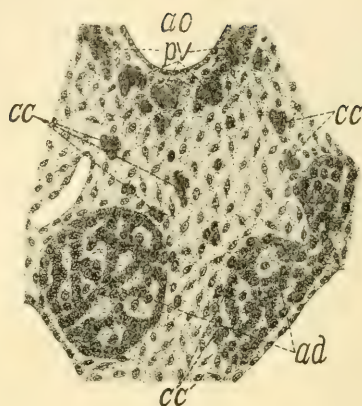
Later development

The record of the later development of the adrenals is essentially a record of the further growth of the glands, the rearrangement of the cells, the adjustment of the chromaffin substance to the cortical substance and the differentiation of the elements derived from the sympathetic nervous system into typical chromaffin cells.

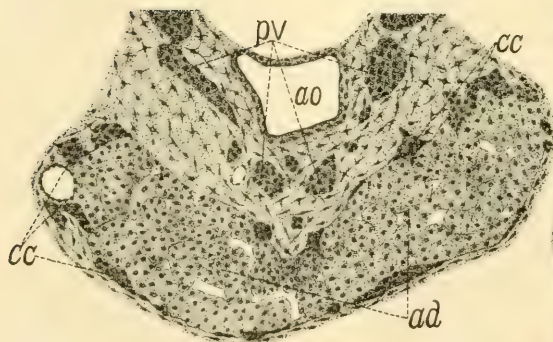
During the later stages of incubation, the development of the adrenals advances comparatively slowly. The adrenal zone be-



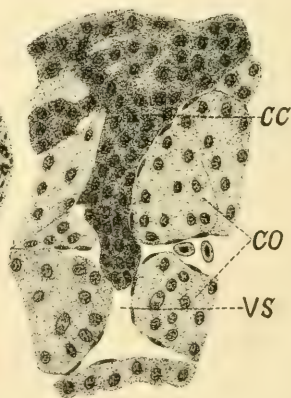
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Fig. 5 Transverse section through the adrenal zone in a thirteen-day embryo of *Thalassochelys caretta*. $\times 150$. *ad*, adrenal anlagen; *ao*, aorta; *pv*, anlage of prevertebral sympathetic plexus; *sy*, anlagen of sympathetic trunks.

Fig. 6 Transverse section through the adrenal zone in a nineteen-day embryo of *Thalassochelys caretta*. $\times 150$. *ad*, adrenal anlagen; *ao*, aorta; *cc*, cells destined to give rise to chromaffin substance; *cc'*, cells of sympathetic origin among cortical cells; *pv*, anlage of prevertebral plexus.

Fig. 7 Transverse section through the adrenals in a twenty-five day embryo of *Thalassochelys caretta*. $\times 100$. *ad*, adrenals; *ao*, aorta; *cc*, chromaffin cells; *pv*, anlage of prevertebral sympathetic plexus.

Fig. 8 Section of adrenal in a thirty-six-day embryo of *Thalassochelys caretta*. $\times 300$. *cc*, chromaffin substance; *co*, cortical substance; *vs*, vascular space.

comes comparatively shorter and the glands gradually assume the general form and location which they maintain during adult life. In embryos twenty-five days old, the adrenals are still closely associated with the anlagen of the prevertebral sympathetic plexuses and a few cells apparently continue to advance from the latter into the masses of cells of sympathetic origin which are becoming approximated more and more closely to the cortical substance. In the anterior region, the right and the left adrenal are distinct and are located approximately at the level of the coeliac plexus which lies between them. Farther posteriorly the right and the left adrenal lie in contact with each other and are apparently connected by a broad bridge of cortical cells; the principal mass of the prevertebral plexuses being embraced in the angle between them (fig. 7, *ad*). Numerous aggregates of cells of sympathetic origin lie in close contact with the cortical substance (fig. 7, *cc*). These aggregates are most conspicuous at the dorsal and the mesial aspects of the adrenals, but aggregates of considerable size may be observed surrounding the entire mass of the cortical substance. The cells of the cortical substance are becoming arranged into more or less distinct aggregates with numerous vascular spaces appearing between them. Not infrequently the aggregates of cells of sympathetic origin penetrate deeply into these vascular spaces, while in sections occasionally such cell-groups occur completely surrounded by the cortical substance.

After the twenty-fifth day of incubation, the association of the cortical and the chromaffin substance becomes more intimate. In embryos from thirty to thirty-six days old, the masses of cells of sympathetic origin have become more conspicuous than in the earlier stages and penetrate more deeply into the spaces between the aggregates of the cortical cells (fig. 8, *cc*). Groups of cells of sympathetic origin which in sections appear to be completely surrounded by the cortical substance are now scattered irregularly throughout the entire section. Such areas in the section probably do not represent groups of cells which have become completely separated from the masses of cells of sympathetic origin aggregated at the surface of the cortical substance, but are, doubtless,

sections of columns of such cells which have penetrated deeply into the vascular spaces between the aggregates of the cortical cells.

In embryos forty-three days old, the adrenals have become more intimately associated with the renal organs and are located on the ventro-mesial surfaces of the latter in approximately the same position which is maintained by them throughout adult life. A larger proportion of the cells of sympathetic origin have penetrated into the vascular spaces between the aggregates of the cortical cells. The distribution of the cortical and the chromaffin

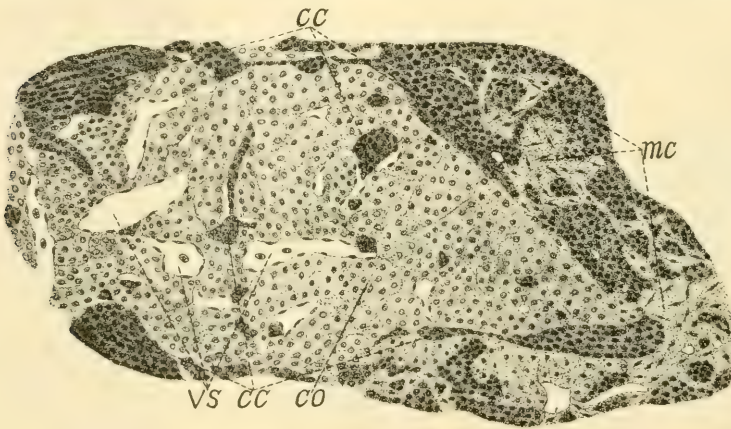


Fig. 9 Transverse section through adrenal in a forty-three-day embryo of *Thalassochelys caretta*. $\times 75$. *cc*, chromaffin substance; *co*, cortical substance; *mc*, mesenchyme cells; *vs*, vascular spaces.

substance, therefore, approaches closely the distribution of these substances in the mature gland. Fig. 9 is introduced to illustrate the distribution of the cortical and the chromaffin substance and their relation to each other in an embryo forty-three days old.

Neither during embryonic development nor in the adult condition do the vascular spaces between the aggregates of cortical cells, into which the chromaffin cells penetrate, show well defined walls which are characteristic of arteries or veins, but are lined by a single layer of flattened epithelial cells (fig. 8, *vs*). That these spaces are blood vessels, however, is indicated by the presence in

them of numerous blood corpuscles. Wherever aggregates of chromaffin cells are found in the cortical substance they occur in or adjacent to such spaces. It is probable, therefore, that chromaffin cells penetrate into the cortical substance only in these vascular spaces.

It may be noted at this point that not all the elements which become differentiated into chromaffin cells ever become incorporated into the adrenal organs. During the later stages of development in embryos of *Thalassochelys caretta* aggregates of these cells may be found entirely apart from the adrenals. In *Chrysemys marginata*, in the adult condition, I have also observed aggregates of chromaffin cells associated with the sympathetic ganglia in proximity with the adrenals. Similar conditions were observed by Poll in *Emys europaea* and by some of the earlier investigators in various species of other classes of vertebrates.

GENETIC RELATIONSHIP AND DIFFERENTIATION OF CHROMAFFIN CELLS

In a series of earlier papers,³ the writer has shown, for the several classes of vertebrates, that the cells which become separated from the cerebro-spinal nervous system and advance peripherally to give rise to the sympathetic nervous system are the descendants of the 'germinal' cells (Keimzellen) of His; viz., the 'indifferent' cells and the 'neuroblasts' of Schaper. The vast majority of these cells (in the lower vertebrates perhaps all of them) advance peripherally as cells of the 'indifferent' type. These cells have the capacity of becoming differentiated into neuroblasts or into embryonic supporting cells. Some of the 'indifferent' cells, however, retain the capacity for further propagation by division and give rise to new generations of 'indifferent' cells after they have become separated from the cerebro-spinal nervous system.

As indicated in an earlier section of this paper, the cells which become separated from the sympathetic anlagen and enter the anlagen of the adrenals there to give rise to the chromaffin sub-

³ See bibliography.

stance are, during the earlier stages of development, identical, as far as may be determined by microscopic observation, with the cells which remain in the sympathetic plexuses. We are forced to the conclusion, therefore, that they are cells of the 'indifferent' type.

The question now arises; why do these cells which become associated with the cortical cells of the adrenals, as well as certain other aggregates which remain apart from the cortical substance, become differentiated into chromaffin cells while other cells endowed with the same initial capacity do not? This question we can not answer conclusively at present. It is suggestive, however, that the differentiation of these cells into chromaffin cells takes place comparatively late in the course of development.

The cells destined to become transformed into chromaffin cells show very little evidence of differentiation before the thirty-sixth day of incubation. Even at the forty-three day stage which was the latest embryonic stage of *Thalassochelys caretta* at my disposal and which falls within a week of the time of hatching, many of the cells of sympathetic origin associated with the cortical substance of the adrenals still remain apparently in their undifferentiated condition. Some of the cells of sympathetic origin in the adrenals, however, have assumed a polyhedral form which is the typical form of the chromaffin cells. The cytoplasm of these cells also stains somewhat more intensely than in the earlier stages, but does not as yet present the coarsely granular appearance, when stained by the iron-haematoxylin method, which is characteristic of mature chromaffin cells.

In several earlier papers ('11 a, '11 b, '11 d), evidence was presented in support of the theory that the processes involved in the peripheral displacement of the cells giving rise to the anlagen of the sympathetic nervous system are stimulated and controlled by the influence of hormones which are produced in the regions toward which the cells advance. The displacement of the cells of sympathetic origin which give rise to the chromaffin cells from the sympathetic plexuses into the adrenals must, doubtless, be accounted for in a similar manner. This displacement can not be accounted for by the mechanical processes involved in growth.

These cells do not advance toward the adrenals in continuous cell-columns, nor do they advance along a single path. Apparently they advance in small aggregates along various paths through a relatively compact mesenchyme. The processes here involved are probably initiated and controlled by the influence of hormones which are produced by the cells of the cortical substance. The differentiation of 'indifferent' cells into chromaffin cells in the adrenals occurs after they have been associated with the cortical substance for a considerable interval. By this time the cortical cells have probably already assumed a secretory function. It is probable, therefore, that the differentiation of 'indifferent' cells into chromaffin cells is also stimulated by the influence of hormones which are produced by the cortical cells. The masses of chromaffin cells which remain apart from the adrenals probably arise from aggregates of 'indifferent' cells whose differentiation into neuroblasts or into supporting elements was delayed, but which were located sufficiently near to the adrenals to fall within the sphere of influence of the hormones produced by the cortical cells.

SUMMARY

1. The anlagen which give rise to the cortical substance of the adrenals arise in embryos of the Loggerhead turtle (*Thalassochelys caretta*) as buds of cells which proliferate from the peritoneal epithelium. The chromaffin substance is derived from the anlagen of the prevertebral sympathetic plexuses. These findings agree with the findings of Poll in embryos of *Emys europaea* and with those of some of the earlier investigators in other types of vertebrates.

2. The cells which become differentiated into the chromaffin cells, like the majority of the cells which advance peripherally from the cerebro-spinal nervous system into the anlagen of the sympathetic nervous system, are cells of an indifferent type; viz., the 'indifferent' cells of Schaper.

3. The processes involved in the displacement of the 'indifferent' cells from the anlagen of the prevertebral sympathetic plexuses toward the adrenals and the differentiation of these cells into

chromaffin cells are probably stimulated and controlled by the influence of hormones which are produced by the cells of the cortical substance.

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THE NERVES OF THE THYROID AND PARATHYROID BODIES

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FIVE FIGURES

Prior to the year 1867 the nerves of the thyroid were usually described as vaso-motor for the supply of the numerous blood vessels.

Peremeschko ('67) examined thin teased preparations of thyroid that had been macerated in acetic acid and water, and found many more nerves than the gland was thought to contain. He described some of these as following the arteries, and others as leaving the vessels, dividing again and again, and finally losing themselves as fine varicosed branches in the interfollicular connective tissue.

Poincaré ('75), after macerating the gland in dilute acetic acid colored with fuchsin, found abundant nerves and nerve plexuses, which he considered to be a separate nerve supply for this organ, connected with the central system by the nerves entering the gland. He also described ganglion cells lying in groups or clumps either in the substance of the larger nerves, in the nerves at their places of branching, or alongside the nerve stems.

Anderson ('94) described the formation of very elaborate perivascular plexuses from which fine fibers penetrate between the follicles and form perifollicular plexuses in which the follicles seem to be imbedded. These nerves are very irregular in their course, do not anastomose, and, after repeated divisions, the terminal fibrillae end in knobs on the bases of the cells. At no time do they enter the cells nor do they penetrate between them.

Berkeley ('95) described perivascular plexuses similar to those of Anderson, and a primary and secondary plexus surrounding

each follicle, the former lying some distance from the bases of the cells, the latter directly on the cells. Small curved branches from the secondary plexuses end in knobs and look as if they penetrate between the cells while others are straight indicating that their endings lie on the basal ends of the cells.

Crissafulli's ('92) description of these nerves agrees in almost all particulars with that of Anderson, with the exception that he found ganglion cells here and there throughout the gland.

TECHNIQUE

The only technical methods for staining nerve fibers that were found of value in this work were the intra-vitam methylene blue method of Ehrlich and the Golgi method.

The methylene blue method was carefully tried in all its forms, but was only valuable in staining the larger nerves accompanying the blood vessels into the gland.

The original Golgi method in the rapid and mixed form together with the modification of Berkeley gave poor results. The most successful and complete staining of the nerves was secured by the use of the 'double impregnation' method of Cajal, modified as to the length of time the tissues were allowed to remain in the different fluids.

This method consists in fixing small pieces of tissue for from six to eight days in a mixture made up of four parts of a 3.5 per cent solution of potassium bichromate and one part of a 1 per cent solution of osmic acid. After fixation and a careful washing in distilled water the tissues are put into a 1 per cent solution of silver nitrate for three days. This is followed by a second fixation for an additional three days, and a second impregnation with silver. After the first day in the second silver solution free hand sections are examined at intervals until the nerves are found to be satisfactorily stained. The tissues are then rapidly dehydrated in several changes of 95 per cent and absolute alcohol, and are quickly imbedded in celloidin, the blocks being hardened in chloroform. Sections, 25 to 100 microns thick, are cut under oil, cleared on the slides in carbol-xylol and xylol, and mounted

in thick xylol-balsam. The slides should then be warmed until enough of the xylol has evaporated to leave the balsam brittle on cooling, and while still warm, covered by warmed cover slips. Preparations prepared in this way are permanent.

THE NERVES OF THE THYROID

The nerves which go to the thyroid are entirely of the non-medullated variety and reach it from the neighboring cervical sympathetic ganglia by following the perivascular connective tissue and the tunica adventitia of the thyroid arteries. It is often very difficult to examine these nerves, for they are obscured in sections stained by the chrome silver method by large amounts of black and brown precipitates, and by the staining of the fat. By carefully examining sections where there is only a small amount of fat, or better, by studying sections stained by the methylene blue method, the nerves accompanying the blood vessels can be followed. They appear as large, wavy, irregular strands, composed of individual varicosed axis-cylinders collected together into bundles similar to the wires in a cable.

These nerves do not branch elaborately outside the gland; the only branches coming from them are small ones which form the perivascular plexuses in the arterial walls, and probably furnish the vaso-motor supply. As soon as the arteries penetrate into the gland substance they branch profusely, this branching being accompanied by a corresponding branching of the nerves, so that as the arteries decrease in size there is a similar decrease in the size of the accompanying nerves. The nerves supplying these smaller arteries (fig. 2) form an elaborate perivascular plexus, and give off the branches which penetrate between the follicles, and form the perifollicular plexuses. The latter are the only ones that can be said to be the true glandular or secretory nerves.

The arterial or perivascular plexuses are formed by the branches of a few relatively large nerve trunks (fig. 2, A), lying in the connective tissue immediately surrounding the vessels, and having a course parallel to that of the arteries. The branching from these nerves is very irregular; the branches taking any direction after

leaving the main trunk. They extend for a longer or shorter distance around the vessels, divide again and again, and finally become resolved into an intricate network of fine end fibrillae. This elaborate nervous plexus is located in the perivascular connective tissue and in the tunica adventitia of the arterial walls, while some of the finer fibrillae penetrate into the tunica media.

The larger nerves are composed of bundles of axis-cylinders, and the branching of these consists merely in some of these axis-cylinders leaving the main bundle and taking a different direction. As this method of branching continues the axis-cylinders finally come to lie singly. From these single fibers are given off true branches, which do not decrease in size but are usually more irregular, more varicosed, and have a more wavy course. The final end fibrillae are often very short, are beset with many varicosities, and soon end in irregular enlargements or end-knobs.

The many crossings and recrossings of the nerves, together with their elaborate branching, is very misleading, and often gives the appearance, especially under low magnification, of true anastomoses. In the thicker sections where the nerves are great in number it is often impossible to distinguish the separate fibers. But if relatively thin sections are examined under high magnification the fibers can all be traced individually. Therefore, contrary to the statement of Berkekey, true anastomoses do not occur. In examining these nerves it is seen that the origin and ending of the most of the branches cannot be traced in a single section for they are only fragments, the remainder having been cut away.

All the nerves that go to make up the perivascular plexuses are more or less varicosed. Even the axis-cylinders that form the large nerve bundles are beset with many irregular enlargements. There may also be precipitations of chrome-silver between the axis-cylinders in places, so as to give the nerves the appearance of solid cords. At the points of branching of all the nerves there are irregular, triangular, black masses, which may be either a portion of the nervous structure or a black precipitate. The smaller nerves, where the axis-cylinders lie singly, are more varicosed than the corresponding parts of the larger nerves; this

being especially true of the final end fibrillae. In fact these nerves appear as if consisting of many irregular enlargements connected by fine threads (fig. 4). The varicosities may be only slightly larger than the fibers or they may be twice as large; the larger ones are cylindrical, spherical, or irregularly triangular, the majority, however, are either oval or spindle-shaped.

The final endings of the nerves consist of fine end branches. The smallest fibers usually divide into two, sometimes more, end fibrillae. The course of these fibrillae is very irregular, they are very varicose and soon end. The tip consists of a spindle-shaped enlargement or end-knob. This ending sometimes occurs in the perivascular connective tissue, sometimes in the tunica adventitia, but most often in the tunica media in relation to the smooth muscle fibers of the vessel walls. Occasionally endings from the perivascular plexuses can be seen penetrating the perivascular connective tissue and terminating in relation with the bases of the epithelial cells of the immediately adjacent follicles.

The plexus surrounding the arteries is more dense and complex than that surrounding the accompanying veins. The venous plexus resembles the arterial in formation and architecture but it is not nearly as elaborate. The peri-capillary plexus is usually formed from the few end branches of a single fiber, running parallel to the course of the vessel.

The most important nerves of the thyroid from a physiological standpoint are those that have their endings in intimate relation to the gland cells, for they are the true glandular or secretory nerves. These nerves pass into the gland substance alongside of, or in the walls of the arteries, together with those that form the perivascular plexuses and furnish the vaso-motor supply. They assist in the formation of the perivascular plexuses but there is no way of distinguishing them from the vaso-motor nerves. There may be, however, a physiological difference in the two kinds of nerves, but if such exists, it cannot be determined histologically.

As has been said before, the nerves do not leave the large arteries and pass directly into the gland substance, for this does not occur until the vessels, by branching, have decreased very much in

size. From the perivascular plexuses of these smaller arteries are given off here and there nerves which penetrate into the inter-follicular connective tissue for a greater or lesser distance and form the perifollicular plexuses. These branches are almost always single fibers, for none of the larger nerves leave the vessels as such.

The perifollicular plexuses completely surround all of the follicles of the thyroid, or, as Anderson has said, there is present a diffuse plexus of nerves in which the follicles seem to be imbedded (fig. 3). The nerves coming from the perivascular plexuses may supply a follicle near the artery, they may supply one at a considerable distance, or their branches may enter into the formation of the plexuses of several follicles. These nerves divide again and again into a large number of fine, varicosed fibers which completely surround a single follicle or adjacent follicles in a dense nervous network. There is absolutely no regularity, arrangement or method in the place or manner of branching, nor in the direction the branches may take after leaving the main stem. Neither is there any regularity in the distribution of the glandular nerves, nor anything that can be said of their distribution, except that they enter into the formation of the plexuses around one or more of the follicles of the thyroid. There are no primary or secondary plexuses, as described by Berkeley, but a single one surrounding each follicle.

In regard to the anastomoses of the nerves of these plexuses the same statement applies that was made in regard to the perivascular nerves. In the thinner sections the nerves can all be traced as individuals, and the branches can be traced to the parent stem if it is included in the section.

The perifollicular nerves are also very varicosed, the varicosities corresponding in size and shape to those found on the nerves of the perivascular plexuses.

Perhaps the most important fact to be determined concerning the nerves of the thyroid is the position and manner of the final endings of the follicular nerves, for the effect of nervous impulses on the secretory activity of the gland cells would depend, to a great extent, on the intimacy of the relations of the nerves to them.

The presence or absence of a definite basement membrane around the follicles is an important point in regard to nerve and cell relations. The majority of investigators are of the opinion that a definitely formed basement membrane is absent, but that there is a condensation of the connective tissue immediately surrounding the follicles, and this gives support to the bases of the epithelial cells (Baber).

The final endings of the nerves are short, fine, very varicosed fibrillae which lie in this condensed connective tissue (figs. 1 and 4). Some of these end fibrillae are curved, as described by Berkeley, while others are straight, and still others, and perhaps a majority, are irregular. They always end in a knob-like enlargement on the basal ends of the cells; each cell, however, does not come into relation with the end of a nerve, but only a few scattered here and there throughout the follicles. I have been unable, by most carefully examining a large number of sections with the best magnification obtainable, to see any of these endings either entering the cells or penetrating into their intercellular substance.

In some of the literature concerning the nerve supply of the thyroid the statement is made that ganglion cells were found in different locations within the gland. Anderson and Berkeley, however, with whom I agree, state that these structures are not present. There are, in many of the sections stained by the chrome-silver method, numbers of black precipitations which resemble ganglion cells very closely in size and shape and could easily be mistaken for them. A careful examination of many of these does not reveal anything resembling a nucleus or nucleolus, while even if nerve fibers enter them, the arrangement is not characteristic of ganglion cell processes.

THE NERVES OF THE PARATHYROID

A careful examination of all the literature accessible concerning the parathyroid has failed to reveal any reference whatever to its nerve supply, and one of the latest histologies makes the statement that this subject needs further investigation.

The technique used in investigating the nerves of the parathyroid was the double impregnation method of Cajal as given in

detail elsewhere in this paper. Several of these small bodies were carefully dissected out and carried through separately, but with poor results. Those sections of the parathyroid in which the nerves are the most abundant are attached to some of the more satisfactorily stained sections of thyroid. The treatment in both cases is, therefore, identical.

The first and most striking thing observed on examination of these sections of parathyroid is the scarcity of the nerves as compared to the great numbers found in the thyroid. It would at first seem that this might be due to incomplete staining, which is not at all probable, for in the adjacent thyroid tissue partially or completely surrounding the parathyroid, the nerves are beautifully stained. The similar density of the two bodies leads me to believe that a method giving a satisfactory result in one would lead to a like result in the other.

The arteries supplying the parathyroid bodies are branches of the thyroid arteries, and may take either or both of two routes in reaching their places of distribution. When the bodies are not closely connected there is one relatively large branch that passes directly into the parathyroid, but when the thyroid partially or completely surrounds the parathyroid, more numerous but smaller branches take origin from the thyroid vessels within the thyroid gland and pass through the intervening small amount of loose connective tissue into the parathyroid. Inasmuch as the nerves follow these arteries, they arise from the large nerve bundles around the thyroid vessels and accompany their branches into the parathyroid. It is very probable, therefore, that the nerves supplying both glands constitute a single set of sympathetic fibers.

Around the parathyroid arteries there are formed nerve plexuses resembling the perivascular plexuses of the thyroid vessels, differing, however, in not being nearly so elaborate, and in consisting of single fibers. The branching of the nerves accompanies the branching of the arteries, so that the smaller arterial twigs usually carry with them a single nerve fiber. There are no nerves around the veins or capillaries.

In most instances the nerves can be traced along the vessel wall, within which are also found their few terminal branches. A few other fibers are present which cannot be followed along a vessel, but which seem to run in the connective tissue between the groups and cords of gland cells. It is probable, however, that these accompany smaller vessels which are too poorly stained to be visible. I am of the opinion that the nerves are entirely vaso-motor for the supply of the blood vessels, and that there are no special glandular or secretory nerves in the parathyroid, because none of the fibers leave the supporting connective tissue and penetrate into the cell groups.

CONCLUSIONS

1. The nerves of the thyroid are entirely non-medullated and reach it from the cervical sympathetic ganglia by following the thyroid arteries.

2. In the thyroid there are formed elaborate nervous plexuses around all the blood vessels and all the follicles, the nerves forming the latter coming from the plexuses surrounding the smaller arteries.

3. The perivascular nerves end in the walls of the blood vessels and furnish the vaso-motor supply, while those of the perifollicular plexuses end on the bases of the epithelial cells and probably carry impulses influencing secretion.

4. All the nerves are varicosed but do not anastomose.

5. The nerves of the parathyroid come from the same set that supplies the thyroid and pass into it along with the branches from the thyroid arteries. These nerves probably all end in the vessel walls and are vaso-motor in function.

6. No ganglion cells are found in either the thyroid or parathyroid bodies.

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All drawings were made with an Edinger drawing apparatus using Leitz compens oculars nos. 4 and 6, and 4 and 16 mm. apochromatic objectives.

Fig. 1 Drawing showing the relation of the nerve endings to the gland cells. $\times 650$.

Fig. 2 A small artery in the interior of the thyroid showing the elaborate perivascular plexus. A, large nerve bundle breaking up to form the plexus; B, nerve leaving the perivascular plexus to form the perifollicular plexus. $\times 630$.



Fig. 3 Several adjacent follicles showing the intimate connections of the adjacent perifollicular plexuses. $\times 250$.

Fig. 4 One of the follicles shown in fig. 3. *N*, endings of the follicular nerves; *V*, varicosities on the nerves; *E*, the epithelial cells, the intercellular substance stained a light brown. $\times 650$.

Fig. 5 Section of the parathyroid. *A*, small artery accompanied by nerves entering the parathyroid from the thyroid; *B*, supporting connective tissue; *C*, nerves in this connective tissue; *D*, nerve endings. $\times 150$.

THE ANOMALOUS PERSISTENCE IN EMBRYOS OF PARTS OF THE PERI-INTESTINAL RINGS FORMED BY THE VITELLINE VEINS

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FIVE FIGURES

While studying the development of the pancreas, Dr. Frederic T. Lewis found two embryos which present anomalies of the intra-embryonic portion of the vitelline veins. He has referred to one of these, a human embryo of 11.5 mm., in Keibel and Mall's Human Embryology (German ed., p. 421); the other, a pig embryo of 10 mm., has not been previously recorded. These specimens, which prove to be of considerable embryological interest, were placed at my disposal, and I have made a careful study of them at the Harvard Medical School, in coöperation with Dr. Lewis, to whom I am indebted for many valuable suggestions. Wax reconstructions have been prepared, both of the abnormal and of normal specimens, which show the course of the veins and the close correlation which exists between their arrangement and the form of the pancreas. The models have been deposited in the collection at the Harvard Embryological Laboratory, where they may be examined at any time.

The peri-intestinal rings formed by the vitelline veins were first made known by His. In a familiar figure, here reproduced as fig. 1, he showed that the right and left vitelline (or omphalomesenteric) veins anastomose with one another at three places, namely (1) ventral to the intestine within the liver; (2) dorsal to the intestine below the dorsal pancreas; and (3) ventral to the intestine above the yolk-stalk. Thus two venous rings are produced, each of which encircles the intestine. He showed, moreover, that the left half of the upper ring and the right half of the

lower ring degenerate, and that the remaining portions of the two vitelline veins form a single vein winding about the intestine.

These relations are correctly represented in fig. 1, but in one respect the drawing of His is subject to criticism. Two vessels are seen ascending along the intestine to fuse at the lower ventral anastomosis. Are these the right and left vitelline veins as Evans has labelled them in his copy of this figure (Keibel-Mall, German ed., p. 653) and as His designated them in the younger 'Embryo R' (5 mm.)? Or is the left vessel, *V.p.* in the figure, the superior mesenteric vein and the right vessel the fused pair of vitelline veins? If the model of His's 'Embryo A' (7.5 mm.), as reproduced by Ziegler, is examined, it will be found that both vessels shown in fig. 1, are continued beyond the loop of intestine along the yolk-stalk, thus representing the right and left vitelline veins respectively. This, however, is an error. The left vessel in an embryo of the stage in question does not extend beyond the intestinal loop. It is the superior mesenteric vein, and the right vessel represents the original pair of vitelline veins, which have fused.

The development of the single stem formed by the vitelline veins may be observed in rabbit embryos. At the time when the lower ventral anastomosis is formed, the yolk-sac is close to the intestine. The veins coming from the right and left halves of the sac meet and anastomose ventral to the intestine and immediately separate to encircle it. With the formation of the yolk-stalk the ventral anastomosis appears to be drawn out in a single stem, which increases in length with the formation of the primary intestinal loop. The prolonged ventral anastomosis becomes separated from the mesentery, so that it appears as a single vein which swings across the abdominal cavity with a peritoneal investment of its own. In this condition, in human embryos of the third month, it was observed by Luschka ('63).

The superior mesenteric vein apparently arises in human embryos of about 5 mm. Thus, in a 4.9 mm. specimen, Ingalls has found several small veins ascending behind the intestine to join the dorsal anastomosis of the vitelline veins, which, it should be noted, is plexiform. These ascending veins probably give rise to the superior mesenteric vein. In a 7 mm. human embryo,

Elze has shown that the superior mesenteric vein is a well-defined stem which empties into the spiral vessel formed from the peri-intestinal rings. The place of junction comes to lie on the left side of the intestine, both in human embryos (Elze) and in pig embryos (Lewis, Thyng). In other words it has shifted ventrally, and the mesenteric vein appears to join the left half of the lower peri-intestinal ring. Thus at the stage shown in His's figure, when the spiral vein has been formed from the peri-intestinal rings, the veins which unite near the intestine are the superior mesenteric vein and the fused vitelline trunk. On the other hand,

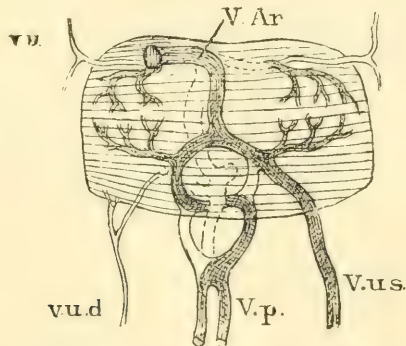


Fig. 1 His's diagram showing the formation of the portal vein, *V.p.* *V.u.* and *V.u.d.*, parts of the right umbilical vein. *V.u.s.*, left umbilical vein. *V. Ar.*, ductus venosus.¹

the place where the right and left vitelline veins unite is near the yolk-sac, as shown in the reconstructions by Lewis and Thyng; and this is far removed from the area included in His's figure.

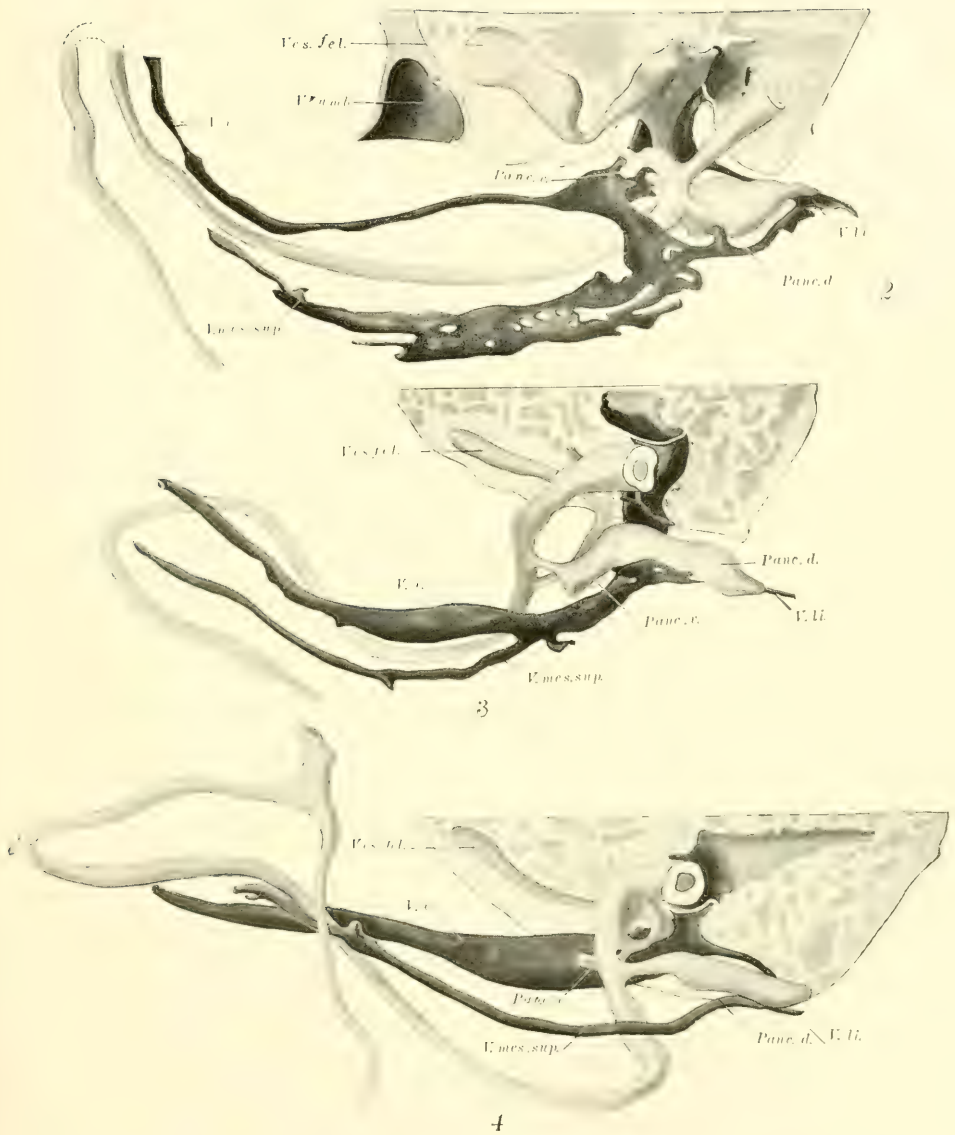
The true relations of these vessels, as here described, have doubtless been well understood by investigators of the venous system, but it is difficult to find an explicit account of them. Luschka recognized a vitelline vein coming from the yolk-sac and a mesenteric vein coming from the mesentery, but apparently he did not consider the possibility that the mesenteric vein might be derived from a left vitelline vein. This possibility, suggested

¹ For the use of the electrotype of this figure, and for many facilities for study and investigation during my stay at the Harvard Laboratory, I am deeply indebted to Professor Charles S. Minot.

by His's models and figures, was rejected by Dexter and Lewis, both of whom figured the elongated ventral anastomosis of the vitelline veins, and portions of the right and left veins of the yolk-sac which unite to produce it. Hochstetter, in his admirable résumé in Hertwig's *Handbuch*, neither figures nor describes the notable elongation of the ventral anastomosis, and Elze fails to recognize it, since he describes the vitelline trunk which crosses the abdomen as the *left* vitelline vein.

With the explanation which we have made, His's diagram (fig. 1) will make clear the nature of the anomaly shown in fig. 2. This figure represents a model of the veins of a pig embryo of 10 mm.; viewed from the left side. In addition to the veins, it shows portions of the stomach and liver, including the gall-bladder, and also the dorsal and ventral pancreases and a large portion of the primary intestinal loop. The distal part of this loop and the yolk-stalk had been cut away before the embryo was sectioned. In reconstructing the organs, only the epithelial portion was included.

In this specimen the fused vitelline veins form a rather narrow vessel showing evidence of atrophy at several points. Within the umbilical cord it occupies a distinct fold of the mesentery. Upon reaching the abdominal cavity the vein leaves the intestinal mesentery and crosses, free from it, to the connective tissue about the duodenum. Ventral to the duodenum it suddenly enlarges and is joined by the superior mesenteric vein. The latter, throughout most of its course, forms part of a net-like system of channels lying in the mesentery. It is a large vein which passes backward and upward in a sweeping curve to join the vitelline vein. In joining the vitelline vein it passes ventral to the intestine instead of dorsal to it, and the main trunk formed by the union of these vessels is on the right side of the intestine instead of on the left. The embryo presents, therefore, a persistence of the right half of the lower peri-intestinal ring, which forms a portion of the main channel to the liver. In the 7.8 mm. embryo described by Thyng, the right half of the lower ring was not found, and it presumably atrophies normally in still younger embryos.



Figs. 2, 3 and 4 Wax reconstructions of parts of the liver, intestine, and adjacent veins. $\times 30$ diam. Fig. 2 Pig embryo: 10mm. Harvard Embryological Collection, Series 1698. Fig. 3 Human embryo: 10 mm. H. E. C., Ser. 1000. Fig. 4. Human embryo: 11.5 mm. H. E. C., Ser. 189. *Panc. d.*, *Panc. v.*, dorsal and ventral pancreases. *Ves. fel.*, gall bladder. *V. li.*, splenic vein. *V. mes. sup.*, superior mesenteric vein. *V. v.*, trunk formed by the fusion of right and left vitelline veins. *V. umb.*, umbilical vein.

The left half of the lower ring normally forms a large vessel which winds around the dorsal wall of the intestine just posterior to the duct of the dorsal pancreas, and then ascends to the liver. The glandular mass of the dorsal pancreas, in growing forward on the right side of the intestine, encounters this vein and becomes molded about it. It sends 'ventral processes' forward, usually on the right side of the vein, but sometimes on its medial side. In the abnormal embryo there are two ventral processes of the dorsal pancreas, both of which are shown in the figure. The normal course of the superior mesenteric vein, after being joined by the vitelline vessel, would be under the duct of the dorsal pancreas and upward on the medial side of these processes, and the shape of the pancreas in the abnormal embryo indicates that such a vessel was present at an earlier stage. It has, however, disappeared and the left half of the lower peri-intestinal ring, together with the dorsal anastomosis of the vitelline veins, is represented by a slender vessel which passes under the dorsal pancreas near its distal extremity. There it is joined by the splenic vein. Before the left limb of the lower ring receives the splenic vein, it presents a small branch directed toward another short branch across the top of the pancreas. These vessels may formerly have connected with one another. The unusual course of these representatives of the dorsal anastomosis of the vitelline veins may be explained by the plexiform nature of the original connection. The upper peri-intestinal ring has developed normally. Its left half has disappeared, and its right half persists as the portal vein.

Finally it should be noted that the ventral pancreas in this embryo is bi-lobed, and that it bifurcates over the upper edge of the abnormal vein. If its lobes correspond with those usually found (cf. Lewis, '11) it is evident that the entire ventral pancreas has been displaced to the right, since the ventral process of the dorsal pancreas approaches its *left* lobe. Its relation to the vein suggests that such a displacement has occurred.

The abnormal human embryo (11.5 mm.), which has been modelled in the same way as the 10 mm. pig, is shown in fig. 4. Above it, in fig. 3, a normal specimen of 10 mm. is presented for

comparison. The smaller embryo is somewhat younger and fails to show the rotation of the intestinal loop, but in regard to the veins the specimens are quite comparable. In the normal embryo the left half of the upper ring and the right half of the lower ring have disappeared. In the abnormal embryo the left half of the upper ring is absent, but the right half of the lower ring remains as the direct continuation of the fused vitelline veins. The ventral portion of the left half of the lower ring has disappeared, but its dorsal portion remains as the continuation of the superior mesenteric vein. Although this anomaly differs from that in the pig in many ways, there is a striking resemblance in the dorsal displacement of the mesenteric vein, which passes beneath the pancreas near its extremity. The explanation of this feature is not apparent.

In the normal human embryo the duct of the dorsal pancreas opens nearer the stomach than the common bile duct. The distance between the two outlets, calculated from the wax reconstruction, is 0.16 mm. In the abnormal embryo, however, the relative position of these outlets is reversed (as already recorded by Lewis) and I find that the duct of the dorsal pancreas opens 0.12 mm. below or caudal to the orifice of the common bile duct. It is possible that the abnormal arrangement of the adjacent veins led to this anomaly, but this cannot be affirmed. The small and rather rudimentary ventral pancreas in the 11.5 mm. specimen extends downward and forward in close relation with the left side of the abnormal vein.

As a summary of the observations which we have recorded, a diagram (fig. 5) is presented, in which the normally persistent portions of the peri-intestinal rings may be compared with the parts found in the pig and in man. In these figures the term portal vein is applied to the vessel formed by the union of the superior mesenteric and splenic veins, in accordance with anatomical usage, and is not extended to include the vessel made by the junction of the superior mesenteric and fused vitelline veins. It would be interesting to find adult specimens which had passed through the abnormal stages figured, but apparently such cases have not been recorded. In the human embryo which we have

described, after the obliteration of the vitelline trunk, essentially normal relations would be restored. But in the pig the superior mesenteric vein would cross in front of the duodenum, and it is probable that this condition will some time be found in adult animals.

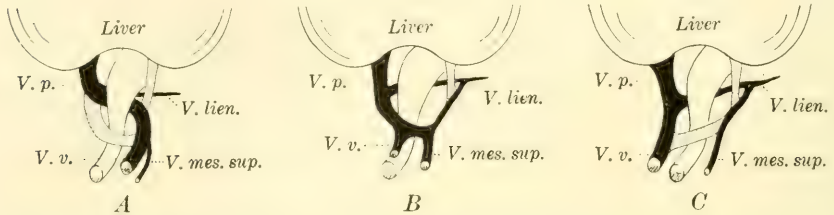


Fig. 5 Diagrams showing, in ventral view, the variations observed in the perintestinal venous rings. The probable position of the obliterated portions is indicated by stippled vessels. *A*, normal human embryo. *B*, abnormal pig embryo. *C*, abnormal human embryo. *V.lien.*, splenic vein. *V.mes.sup.*, superior mesenteric vein. *V.p.*, portal vein. *V.v.*, trunk formed by the fusion of the vitelline veins.

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THE DEVELOPMENT OF THE AORTA AND AORTIC ARCHES IN RABBITS

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NINE FIGURES

The development of the primary blood-vessels in the body of the embryo has for many years been a matter of dispute. Evans, in the German edition of the second volume of the Keibel-Mall Embryology, sums up the matter as follows:¹

Whether the first blood-vessels of the embryonic body arise by ingrowth from the yolk-sac capillaries, or whether the embryonic vessel-stems, or at least a part of them, originate in situ from the mesoderm of the body, is still an open question. Both views have found their supporters; the name of His is connected with the first mentioned idea, the names of Rückert and Mollier especially with the second.

In birds it is possible to prove that the greater part of the descending aortae develop from the mesial border of the capillary plexus which has extended in from the yolk-sac, and this is very probably true of mammals also; but (to quote again):

For the cranial part of the aorta, on the other hand, the results are contradictory. His describes it as arising from a further ingrowth of the same extra-embryonic capillaries which form the aorta in its more caudal portion; the capillary chain grows finally over the blind end of the pharynx, turns ventral-ward, and joins the cranial part of the heart cavity. In rebuttal, Rückert and Mollier have stated in numerous articles that the aortae arise in loco from cells of the visceral layer of the mesoderm. It is impossible at present to insist that the anlagen found on the yolk-sac are the only ones for the endothelium of the body vessels. (Keibel-Mall *Entwicklungsgeschichte*, vol. 2, p. 552, etc.)

¹ In the American edition of this work some of the results of the present paper have been added.

In this paper and by means of the following reconstructions made from serial sections of embryos in the Harvard Embryological Collection, I hope to show clearly that the view of His and his supporters is in the main correct, that the cranial part of the aorta arises as an extension of the capillary network of the yolk-sac; and also to throw more light on the development of the ventral aorta, the aortic arches, and the pulmonary artery. For the study of this question I have chosen to work primarily with the rabbit, partly because of the excellence of this material in this laboratory, and partly because the presence of the 'lateral hearts,' described by Rathke, and easily recognizable in this species even in early stages, readily marks the position of this part of the blood-vessel net, and makes interpretation of the secondary foldings much simpler.

To some extent this same material was used by Dr. F. T. Lewis in a paper on "The Intra-embryonic Blood-vessels of Rabbits from $8\frac{1}{2}$ to 13 days," which, accompanied by a demonstration of sections and graphic reconstructions, was read at the meeting of the American Association of Anatomists in 1903, but never published in full. In the report of the Proceedings in *The American Journal of Anatomy*, vol. 3, a résumé is given as follows: "From the network of vessels in the splanchnopleure of the yolk-sac, all intra-embryonic vessels are apparently derived as offshoots. The network ends mesially in the two aortae. With the formation of the pharynx, this net is so folded as to produce dorsal and ventral aortae with the connecting first arch." It will be seen that Lewis agrees with His as to the origin of the dorsal aorta, but discards the idea that this vessel grows forward around the tip of the pharynx to join ventrally with the anterior end of the heart. A glance at figs. 1 and 3 will show that this is correct; dorsal aorta, first arch, ventral aorta and heart anlage are all laid down almost simultaneously.

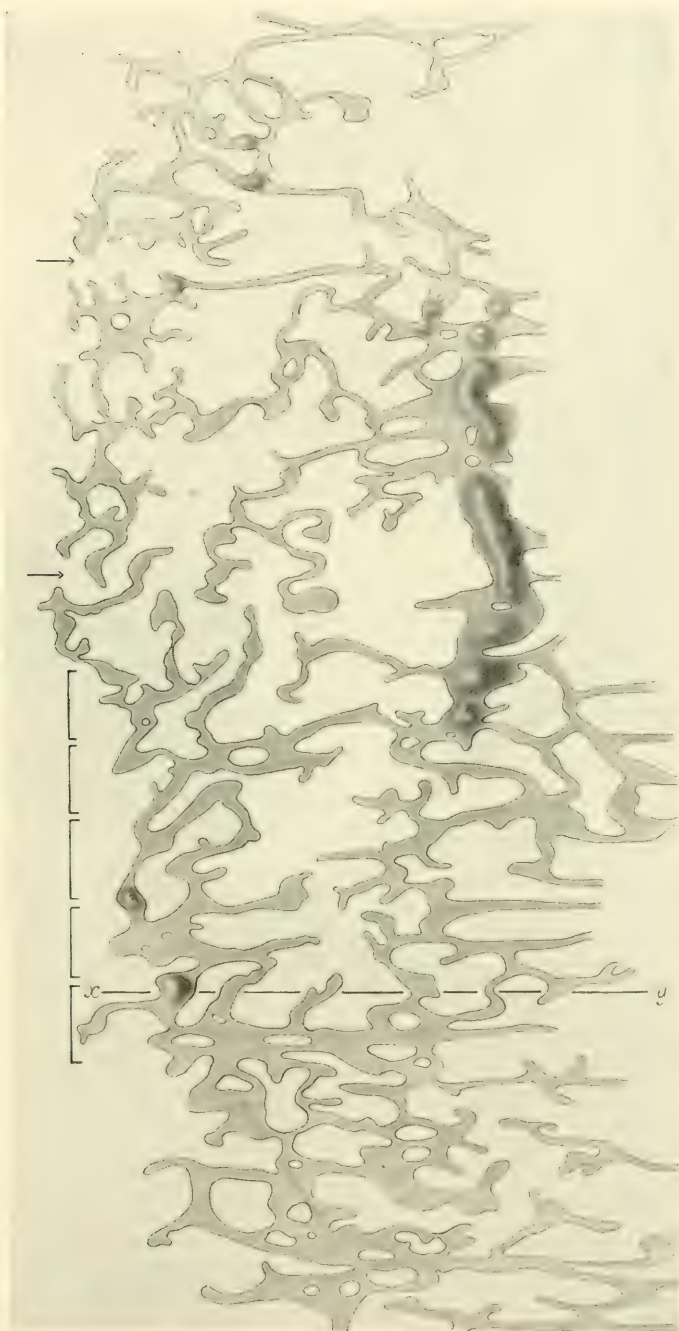
Here a few words are needed on the character of the early blood-vessels. The most recent investigations in this field have been carried on almost exclusively by careful injections of fresh embryos, which are then studied as transparent objects or are converted into sections from which reconstructions are made. This method

presupposes that all vessels are injectable, and in fact the claim is made that many collapsed vessels cannot be distinguished in sections until opened and marked by the injection mass. While in no way wishing to belittle the value of this method of research, or to discourage the increase of the many beautiful and valuable preparations obtained by its use, I still feel that its limitations, as they are shown in this paper, should be pointed out. As insisted on by His in 1900, and by many other authors (His' name is used as that of the champion of this idea), the first blood-vessels on the yolk-sac and elsewhere are solid cords or strands of cells, without lumen: or to use other words, the actual vessels are always preceded by solid growths, which secondarily become hollow and form vessels. These solid growths, for which I wish to propose the term 'angioblast cords,' usually take the form of nets, which may persist until the separate strands are hollow, as shown by the injections of Evans and others, or may, as I hope to show, disappear in part without ever becoming injectable. The two views are clearly shown by a comparison of two figures, one from His ('00, 2, fig. 91), the other from Evans ('09, figs. 1, 2, 3) both showing the caudal end of the aorta of a chick embryo; by the injection method the capillary network is revealed, while His represents a network of solid sprouts preceding the hollow vessels. In this case, since an injection of these so-called solid sprouts would give practically the same picture as would be obtained if they were not seen and so left out of the drawing (the network being similarly placed throughout), we have no direct proof that the sprouts are not potentially hollow, or in other words merely collapsed; but in the development of the anterior part of the aorta there are nets of solid angioblast cords present at an early stage, parts of which have certainly never been shown by injections, and may therefore, for the present at least, be considered solid. Here and there in this solid network there are hollow spaces, or true vessels, unconnected at first with one another and with the lateral capillary net except by the solid angioblast cords, and therefore not to be reached by any injection mass from this lateral net; for such unconnected hollow spaces I suggest the term 'angiocysts.' Thus the angioblast cords retain certain char-

acteristics of the blood-islands, in that they also change from solid to hollow independently; but in the angioblast cords there is no sign of the formation of blood-cells. It was the observance of these isolated spaces, which later fuse to form large vessels, that lead to the often repeated statements of Rückert and Mollier and others that the dorsal aortae arise in situ from the cells of the mesoderm; and in truth the connection with the lateral capillary net is short lasting and sometimes extremely tenuous. Türistig ('84, 1) recognized the presence of solid cords leading a short distance from these hollow spaces, but did not trace their connections; others of this school have missed them entirely.

The story of the development of the primary arterial system can best be told with the aid of the figures. Fig. 1, a reconstruction of the angioblast cords of one side of a rabbit embryo of five segments, shows these cords, streaming in from the network over the yolk-sac, the cut ends where the reconstruction was discontinued showing at the right border of the figure. They have grown from right to left of the figure, occasionally anastomosing, until near the median line, which lies at the left of the figure. The shape of the meshes of this net indicates, it seems to me, the direction of this growth, and the rapidity with which it has occurred. Cephalad the net is limited by the proamnion, where no mesoderm exists; caudad the net continues beyond the portion drawn. In a few places the cords have become hollow; here and there near the median line, and especially at the right of the figure, where a considerable chain of hollow spaces extends longitudinally, near the lateral border of the embryo proper. This chain, the future lateral heart, lies beneath the coelom, and like the coelom at this stage is situated only in the anterior third of the embryonic body. At the left of the figure the net ends rather

Fig. 1 Rabbit, $8\frac{1}{2}$ days, 5 segments, 3.4 mm., H.E.C. no. 650. Reconstruction of the angioblast of the left side, anterior two-thirds of the embryo, seen from the entodermal cavity. The median line of the body is to the left of the drawing, solid cords of angioblast come from the yolk sac on the right. Hollow vessels are indicated by the shading. Brackets show position of somites; arrows mark regions where the aortic network is as yet incompleté; $x - y$ indicates plane of section of fig. 2. $\times 150$.



regularly, and the meshes are slightly smaller, especially toward the head. Closer examination will show that each cord extends from the lateral heart almost directly toward the median line, then suddenly spreads longitudinally, as though its further direct course were blocked by some obstruction. In all but two places, which are marked by the arrows, the longitudinal strands of this mesial net have anastomosed with others; at these two places the longitudinal network is interrupted, and we may see clearly that this part of the net, the future dorsal aorta, is an ingrowth from the lateral vessels.

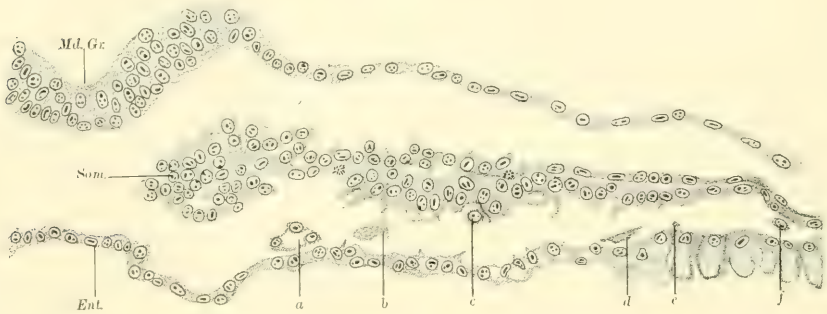


Fig. 2 Rabbit, $8\frac{1}{2}$ days, H.E.C. no. 650, section 317. Section through the plane $x-y$ in fig. 1. *Md.Gr.* = medullary groove; *Som.* = 5th somite; *Ent.* = entoderm. *a, b, c*, etc. = sections of the angioblast network. $\times 175$.

If we examine fig. 2, a section of the same embryo, we can see these cords in their relation to the germ layers. The embryo has become shrunken in preservation, so that the layers are separated slightly from one another. The angioblast cords, indicated by the small letters, are seen to adhere now to the entoderm, now to the mesoderm, a fact which made them hard to follow, but which is obviously the result of this shrinkage. Let us imagine that the layers are close together. It will be seen that the somite will then touch both the ectoderm and the entoderm; it is this that forms the obstruction to further growth of the angioblast cords toward the median line. Where the somite does not exist, as in the head region, the cords extend further, and are then halted by the closely approximated medullary groove and entoderm;

this can be seen by noting the position of the somites in fig. 1. Just lateral to the somite is the thin neck of mesoderm, the nephrotome, which coming between two thicker portions, will be held above the entoderm, to form the roof of a small longitudinal canal; it is in this canal that the angioblast cords have room to expand and become hollow (fig. 2, *a*). In the head region, a similar though broader canal is provided by the uplifted lateral edge of the medullary groove; and in this region the reconstruction shows that the hollow parts of the net are found more widely distributed; in fact their chain follows forward the line of the spreading edges of the medullary groove. Thus, as in the case of the vertebral arteries, a longitudinal trunk is made by the anastomosis of branches from transverse vessels.

Another place where the blood-vessels have an opportunity to develop freely is beneath the coelom. At this early stage the coelom is almost exclusively represented by the amnio-cardiac vesicles, which lie cephalad to the level of the somites. Here is seen the development of the chain of spaces which is to become the lateral heart; the extension caudally of this is limited by the absence of the coelom.

Here then, in this early stage of the embryo before the pharynx has begun to be formed, we see a flat sheet of angioblast cords, forming a network, lying between entoderm and mesoderm, derived as an extension from the lateral extra-embryonic area. Its growth is limited cephalad by the absence of mesoderm in the proamnion; mesially by neural groove or somite. It occupies two areas especially where further growth is possible, namely under the nephrotome or the angle of the medullary groove, and under the amnio-cardiac vesicle.

Turning now to the next older embryo, one of six or seven segments, figs. 3 and 4, we can see that two changes have taken place; a portion of the net has vanished, and the remaining portions include more hollow vessels, which are still, however, connected by solid angioblast cords. The persistent parts of the net are exactly those indicated in the last paragraph as occupying favorable positions; beneath the coelom lies the lateral heart, and beneath the edge of the neural groove lies the dorsal aorta. This

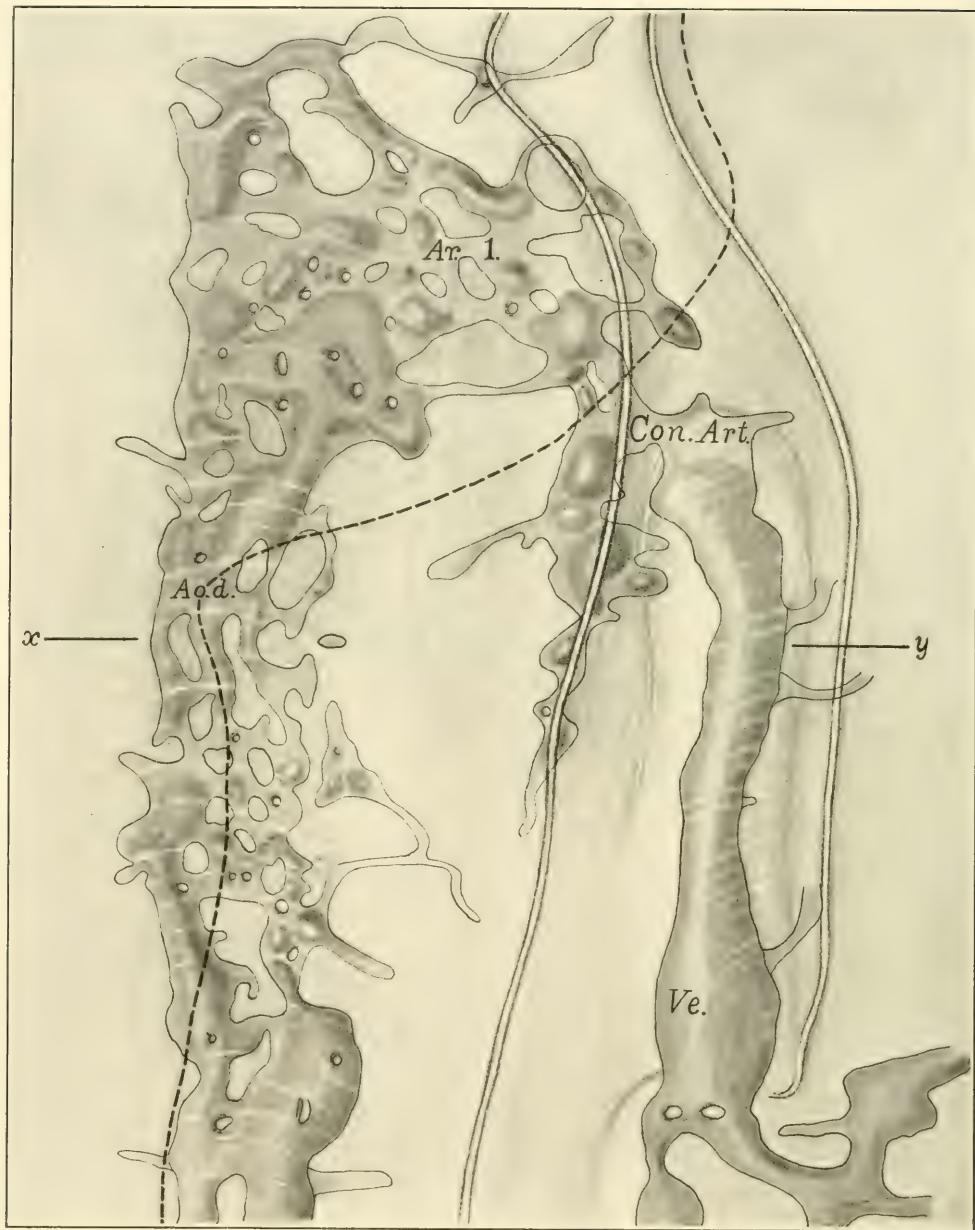


Fig. 3 Rabbit, $8\frac{1}{2}$ days, 6 to 7 segments, 3.4 mm., H.E.C. no. 624. Reconstruction of the angioblast of the right side, anterior third of the embryo, dorsal view. Orientation as in fig. 1. The coelom is represented as opened to show the lateral heart within. *Ao.d.* = network to form the dorsal aorta; *Ar.1* = first aortic arch; *Con. Art.* = conus arteriosus; *Ve.* = venous end of lateral heart, with vitelline vein entering it. *x-y* indicates plane of section of fig. 4. Broken line marks limit of medullary groove. $\times 200$.

reconstruction does not include the region of the segments. Cephalad, this raised edge, the top of which is indicated by the dotted line, makes a wide sweep laterally to form the future optic vesicles, and extends over the region of the coelom; so that a wide portion of the net may remain here, and may join with the vessels under the coelom. Thus the first aortic arch is formed, a net of vessels and cords connecting the dorsal aortic net with the anterior end of the lateral heart.

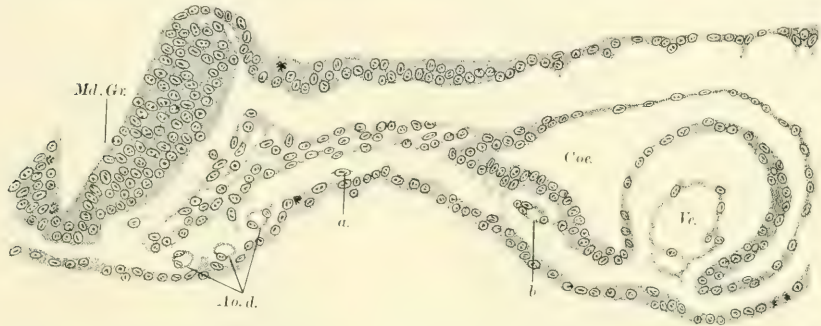


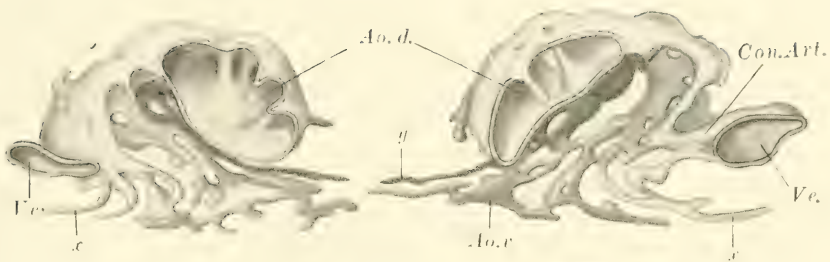
Fig. 4 Rabbit, 8½ days, H.E.C. no. 624, section 101. Section through plane $x-y$ in fig. 3. *Md.Gr.*, medullary groove; *Coe.*, coelom; *Ao.d.*, three strands of network for dorsal aorta; *a.*, isolated portion of angioblast; *b.*, part of network; *Ve.*, lateral heart. $\times 175$.

Of the original net of angioblast cords between the lateral heart and the aortic net almost nothing remains. Here and there a few isolated cells can be found, not connected with the rest of the network; one of these is shown in fig. 4, others, though recognized, were left out of the reconstruction for the sake of clearness. Other cords have also been lost; of those connecting the lateral heart with the extra-embryonic angioblast only the most caudal remains, the others have either entirely disappeared or have lost their lateral connections. The cause of this latter destruction of the net is shown in fig. 4; the lateral heart, covered by a reflection of the mesoderm, has expanded so far into the coelom that vessels from it to the lateral net must take a curving course, and would probably be compressed between mesoderm and entoderm. This has occurred progressively from before backward, until the lateral heart gradually leaves the region of the coelom; here no

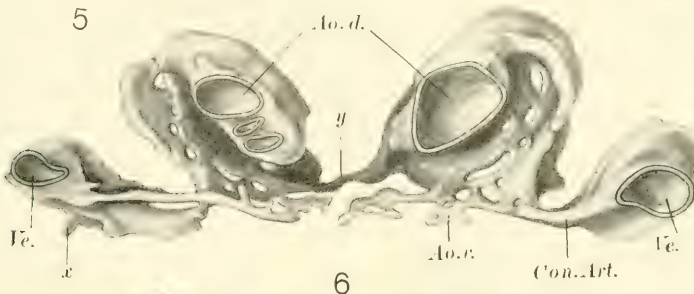
such influence is brought to bear on the angioblast cords, and here they enlarge and become the vitelline veins.

Another agency at work in the further development of these blood-vessels is shown in fig. 4. The shape of the layer of entoderm indicates a longitudinal folding of this layer to form the pharynx, of which a point near 'a' is to be the lateral edge, and a union of the layer near 'b' with a similar point on the opposite side of the embryo is to complete the floor. This fold ends anteriorly by curving to the median line under the network of the first aortic arch. Thus the pharynx is due, in the rabbit at least, not so much to the usually described pouching forward of the entoderm as to a lateral folding of the layer, and the floor of the pharynx is completed by a union of the entoderm of the two sides, which soon fuses and forms two continuous sheets, one for the floor of the pharynx, the other for the upper wall of the archenteron.

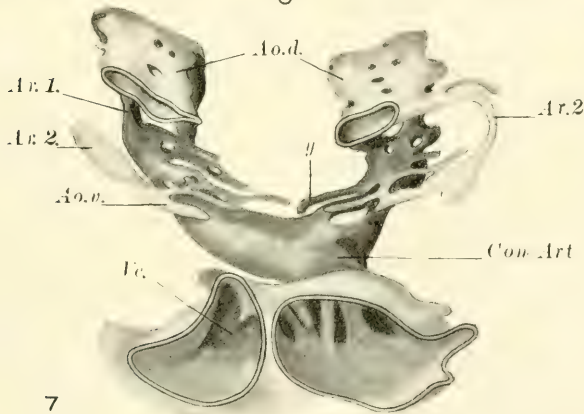
This folding in a more advanced stage is shown by the shape of the blood-vessels in figs. 5 and 6, since the vessels always lie close to the entoderm. The dorsal aortae, still showing, by their frequent subdivision, signs of their origin from a network of vessels, are in the same relative position as before, dorsal to the entoderm of the pharynx. The lateral part of the network has been rolled in underneath the pharynx, whose crescentic outline is marked by the plexus of vessels which forms the first arch. By this folding or rolling in process the lateral edge of the network now lies beneath the pharynx and near the median line, and as the mesoderm makes its way between the floor of the pharynx and the roof of the archenteron, new shoots from these vessels pass toward the median line, and may even anastomose with others from the opposite side. This ventral plexus of vessels, many of which are at first solid cords, is the first indication of the ventral aorta. It is connected with the lateral heart, as can best be seen in fig. 6, by means of a slender vessel, the conus arteriosus, or bulb; and the lateral heart has, so to speak, lagged behind in the folding, so that the curve of the blood-vessels and of the entoderm in transverse sections of the embryo makes an *S*, the upper curve of which comprises the dorsal aorta, first arch, and ventral aortic



5



6



7

Fig. 5 Rabbit, 9 days, 8 segments, 3.2 mm., H.E.C. no. 621. Reconstruction of the blood-vessels of the head end of the embryo; seen as though looking forward from the anterior end of the lateral hearts, to show network for first arch. *Ao. d.*, dorsal aortae; *Con. art.*, conus arteriosus; *Ve.*, lateral hearts; *Ao. v.*, network for ventral aorta; *y*, extension toward median line; *x*, blind ends of obliterated vessels from yolk-sac. $\times 125$.

Fig. 6 Rabbit, 9 days, 8 segments, 3.2 mm., H.E.C. no. 709. Reconstruction and lettering similar to those in fig. 5. At *y* the extension of the ventral aortic network has fused with that of the other side. $\times 125$.

Fig. 7 Rabbit, 9 days, 11 segments, 3.8 mm., H.E.C. no. 619. Reconstruction and lettering similar to those of fig. 5. *Ar. 1.*, *Ar. 2.*, first and second aortic arches. $\times 125$.

plexus, while the lower curve includes conus arteriosus and lateral heart. The upper curve corresponds with the pharynx, the lower is below the pharynx and associated with the coelom.

If we notice the position of the lateral heart in figs. 4 to 7 we can see it is gradually rolled over and inverted, at its anterior end, so that whereas in fig. 4 it projected dorsally into the coelom and therefore was connected with the other vessels ventrally only, in fig. 6 the projection is distinctly lateral. In fig. 7, from a rabbit of eleven segments, the lagging lateral hearts have ultimately met in the median line and partly fused, but not until the pharynx floor has been completed and the ventral aortic network established, so that the inverted and fused lateral hearts lie at a distinctly lower level, connected with the ventral aortae by the ascending conus arteriosus, which itself is composed of two fused halves. The blood-vessels of this part of the embryo now lie in three tiers or levels; dorsally, the dorsal aortae, ventrally the heart, and between these two the ventral aortic plexus, joined to the former by the first arch and to the latter by the conus arteriosus.

On the recognition of this middle tier, the plexus of the ventral aortae, depends the proper understanding of the development of the rest of the aortic arches and of the pulmonary arteries. If we turn back to fig. 3, we shall see that the three levels are already indicated in the angioblast net; the dorsal aorta, in plexus form, occupies a distinct region toward the median line, the lateral heart lies toward the right of the figure, while, connected with this by the conus arteriosus and with the dorsal aorta by the first arch, a portion of the net remains, expanded longitudinally, and lying beneath the mesial border of the coelom. This is to form the ventral aorta, and is to lie in the floor of the pharynx, while the conus is to lead from this level to the heart, which occupies a more ventral position.

It will be noticed that in fig. 3 there is an extension of the ventral aorta caudad from the conus arteriosus. This may be precocious in this case, for in the two embryos of eight segments, figs. 5 and 6, no such extensions were found, while in the embryo of eleven segments it is again present. This caudad growth of the ventral aortic net lies in the thin sheet of mesoderm between the

floor of the pharynx and the pericardial cavity, and ultimately reaches the level of the pulmonary anlagen.

THE AORTIC ARCHES

The first aortic arch has already been described as a persistent portion of the original angioblast net, folded around the anterior end of the pharynx. The cords of this net become hollow, as is shown by Evans in the drawing of the injected vessels of a duck embryo (Keibel-Mall, vol. 2, fig. 398). Later, as is seen in figs. 7 and 8, the net becomes reduced to two vessels on each side, and in the rabbit frequently breaks up into capillaries before being reduced to a single trunk.

The second aortic arch (figs. 7 and 8) arises as a lateral extension from the plexus of the ventral aorta, frequently double on one or both sides, consisting at first of solid cords and hollow spaces, and met by much shorter outgrowths from the dorsal aorta. The potentially double character of this arch, even after it has attained a considerable development, is shown in fig. 8, *x* and *y*. While the second arch is becoming established the plexus of the ventral aorta is extending still further caudad, and again giving off lateral branches, which run between the entodermal pouches to the dorsal side of the pharynx, and again are met by shorter growths from the dorsal aorta. Thus the third and fourth arches are formed and, as a glance at fig. 8 will show, they also, from their plexiform origin, are potentially multiple on each side.

With the growth of the pharynx the conus arteriosus, which joined the ventral aortae originally cephalad to the second arch, has moved caudad, and later opens almost directly into the third and fourth arches. This condition is represented in fig. 9. Here still we see the plexus of the ventral aorta extending caudad from the ventral part of the fourth arch, as it did earlier from the second arch. Its plexus formation is easily recognized, as it lies in the thin sheet of mesoderm between the pharynx and the pericardial cavity, but it no longer crosses the median line on account of the presence of the keel-like growth of the pulmonary anlage from the ventral wall of the pharynx. From the dorsal part of

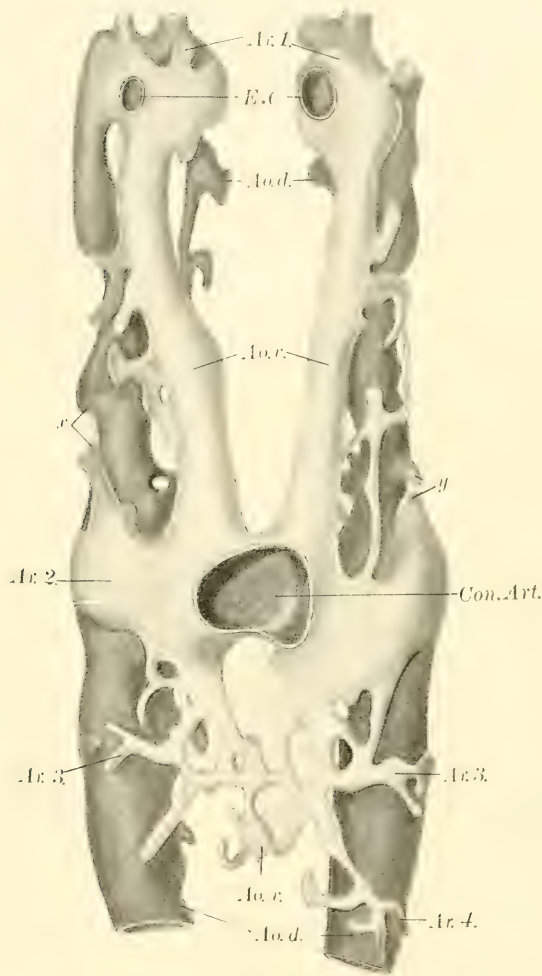


Fig. 8 Rabbit, 10 days, 23 segments, 3.2 mm., H.E.C.; no. 940. Reconstruction of the blood-vessels of the head end of the embryo, seen from the ventral side; the heart removed by cutting through the conus arteriosus. Lettering same as in fig. 5, and also *Ar. 1*, *Ar. 2*, *Ar. 3*, *Ar. 4*, aortic arches; *E. C.*, external carotid arteries, cut; *x* and *y*, remains of second channel for second aortic arch. $\times 125$.

the fourth arch and from the dorsal aorta sprouts arise, at first in part solid, which curve around the pharynx and, entering the same sheet of mesoderm in which the ventral aorta lies, form there,

on the left side of the embryo, an anastomosing plexus. On the other side of this embryo these sprouts from the dorsal aorta are much simpler, though still double, while a lateral extension of the plexus of the ventral aorta replaces that derived, on the left, from the dorsal sprouts. In other embryos there is great variation in these vessels. There can be no doubt that these vessels from the dorsal aorta are about to join the ventral plexus and form another arch; nor can there be any question that in this case there are two

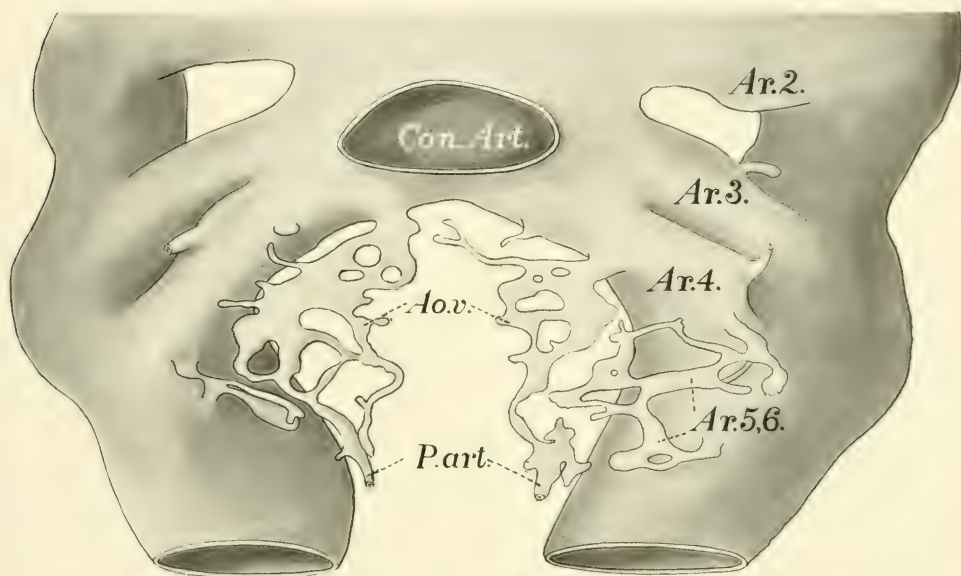


Fig. 9 Rabbit, $10\frac{1}{2}$ days, 3.2 mm., H.E.C., no. 560. Reconstruction and lettering similar to those of fig. 8. *P.art.*, extension of ventral aortic network as pulmonary arteries. $\times 125$.

channels on each side. While not wishing to go deeply into the controversy over the presence or absence of a sixth aortic arch, I may say that it seems to me that the final solution should come from a further study of the entodermal pouches, of the branches of the nerves, and of the cartilages in this region. We have seen that the four first arches are potentially double on one or both sides, and we now find that the last arch is so also, with, as is

known, great variation in the points of origin and forms of anastomosis. We know that the fourth pouch is forked at the end, but so is also the third pouch. Branches of the vagus have, in a few instances, been found which seem to indicate the presence of an extra arch; but aberrant nerve branches are not unknown elsewhere. As far as the early development of these vessels is concerned, there is nothing certainly to prove the presence of an interpolated arch.

THE PULMONARY ARTERY

It will be noted that the sprouts for this last arch arise chiefly from the dorsal vessels, instead of from the ventral net. I also wish to point out that the net grows beyond the arch, before the arch has become complete. In other words this extension of the ventral aortic net forms well defined pulmonary arteries, one on each side, before the pulmonary arch exists; the pulmonary artery is in no sense a branch of the pulmonary arch, and moreover, in the strictest sense, the arch extends only from the dorsal aorta to the pulmonary artery, the ventral part of the vessel usually called the arch is really the ventral aorta. The persistent pulmonary arteries are entirely ventral; they have been joined during embryonic life by branches from the dorsal aorta, but such branches are only temporary.

Here I must add a few words in regard to some recent statements on the development of the pulmonary arteries. Evans ('09, fig. 21) gives a figure of the injected pulmonary arteries of a pig embryo of 12 mm., and in the German edition of the Keibel-Mall Embryology he copies (fig. 396) a figure of Fedorow showing the pulmonary arteries of a guinea-pig embryo of twenty-one days. In both cases the vessels form a narrow plexus in front of the trachea, and Evans² concludes that here, as in other growing vessels, the main trunks are preceded by a capillary net. That he is correct in the main, that the pulmonary arteries do arise as the extension of the net of the ventral aorta, we have just seen;

² Dr. Evans has very readily and kindly acknowledged his error to me; Fedorow did not make the misinterpretation.

but these plexuses of the pulmonary arteries across the median line are of later occurrence, after much mesoderm has grown in between the trachea and the pericardial cavity. It is unfortunate that the pig and the guinea-pig were chosen as illustrations, for, as I have shown in previous papers ('02, '09), in these two species only, so far as I am aware, is such a secondary net found. After the formation of this net one of the connections with the pulmonary arches is lost, so that in these two embryos the trunk of the pulmonary arteries seems very long before the right and left branches are given off. In those two papers I spoke of the pulmonary arteries as branches of the pulmonary arches, a statement which I am now very glad to correct.

SUMMARY

In the rabbit, the dorsal aorta, the first aortic arch, the conus arteriosus and the lateral heart are all parts of an original network of angioblast cords derived from the extraembryonic plexus of blood-vessels. Those portions of this network which are mechanically favored in their position persist; the other portions disappear. The favored portions lie (1) under the coelom, (2) under the nephrotome, or (3) under the raised edge of the medullary groove. The connection between first arch and lateral heart is permitted by the lateral expansion of the medullary groove which extends over the coelom.

This net of angioblast cords is folded in the formation of the pharynx, so that its lateral edge, anterior to the lateral heart, becomes the ventral aorta. In this folding the lateral heart is retarded, and thus comes to lie on a lower plane, more ventral. The connection between the two planes is by means of the conus arteriosus.

From the net of the ventral aorta a plexus grows mesially and caudally, still forming the ventral aorta. Lateral growths from this pass around the pharynx, often in plexus form, and make the second, third and fourth aortic arches. The conus arteriosus is moved caudad to a position opposite the fourth arch.

A further extension of the plexus of the ventral aorta, situated between the floor of the pharynx and the dorsal wall of the peri-

cardial cavity, but prevented from crossing the median line by the presence of the median pharyngeal outgrowth to form the trachea, extends to the lungs as the pulmonary arteries, which are later joined by vessels springing from the dorsal aortae. These vessels, which may be double and plexiform, constitute the fifth (and sixth) arch.

Although dealing in this paper with the development as found in rabbit embryos, I have examined various other species, as chick, pig, sheep, etc., and feel satisfied that in all essential points the story of the development of these primary vessels in other vertebrates will be found similar to that here described.

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THE BEHAVIOR AND RELATIONS OF LIVING CONNECTIVE TISSUE CELLS IN THE FINS OF FISH EMBRYOS WITH SPECIAL REFERENCE TO THE HISTOGENESIS OF THE COLLAGINOUS OR WHITE FIBERS

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TEN FIGURES

In the process of connective tissue development the cells first arise, the fibers later appear. This sequence is established beyond controversy. The ontogenetic relation of cell and fiber is not, however, so thoroughly established. The theories advanced may be grouped under three heads: (1) Intra-cellular origin, the cells may transform into fibers (Schwann, Valentin, Boll, Flemming, Spuler, Livini). (2) Extra-cellular origin, the fibers arise in the intercellular substance by its fibrillation, or possibly as a secretion from the cells (Henle, Merkel, Virchow, Kölliker). (3) Epicellular origin, the fibers form in an ectoplasm at the surface of the cell (Schultze, Hansen, Golowinski, Mall).

These theories have all been primarily founded upon the results of examination of 'fixed' or 'killed' tissue, or upon the study of fresh teased tissue. Living connective tissue has been studied in the mesentery of the frog and other animals under conditions which are accompanied by marked inflammatory reaction and certain stages of the formation of exudates and of scar tissue have been thus investigated, and more recently movements of connective tissue cells have been observed in tissue cultures but so far as I know the theories of the histogenesis of connective tissue have not been examined with reference to the behavior of living cells under normal conditions.

Living connective tissue cells have been seen in tissue cultures by Harrison, Burrows, Carrel and Burrows, Margaret R. and W. H. Lewis and others, to exhibit a certain motility, and Harrison has recently emphasized the stereotropic tendency of connective tissue cells in cultures when in contact with foreign surfaces, glass, spider-web, etc. But so far as I know, the histogenesis of connective tissue fibers has not been so studied, and at best the culture method is open to some criticism on the ground that while the connective tissue cells are undoubtedly alive and active, yet they exist under very unusual, if not abnormal, conditions whose effects have not yet been subjected to complete analysis. Under these conditions the behavior of the connective tissue elements while probably similar, is not certainly in exact conformity with that of the tissue within the embryo.

In order that deductions based upon these several methods of examination be adequately controlled it appeared desirable that developing connective tissue be studied in the living animal under conditions which were in every respect normal, or which, at least, resulted in no inflammatory reaction. In mammals this endeavor is fraught with considerable difficulty owing to the size of the mammalian embryo and the depth beneath other tissues, often not transparent, at which the connective tissue lies.

During the past summer I had the opportunity, through the courtesy of the Marine Biological Laboratory at Woods Hole, of studying connective tissue in the fins of living fish embryos under conditions which were wholly normal and unaccompanied by any evidence of inflammatory reaction.

If a free swimming *Fundulus* embryo is placed on a hollow ground slide it will continue to swim, often actively, and its heart beat and circulation are maintained. It may be observed for some minutes and at the end of observation may be returned to the aquarium to continue an uneventful existence for hours or days thereafter. If a drop of chlorotone is added, or frequently without its addition, the fish will remain quiet for some minutes, thus permitting continued observation of connective tissue cells in his semitransparent fins. Certainly cells studied under these conditions are open to no criticism of abnormality.

The viability of the animals is unaltered for I have kept them for several days after such observation without any indication of decreased activity on their part. Even embryos which have been quieted by chlorotone, as well as those immersed for hours in a solution of Bismark brown in sea water, I have resuscitated and kept alive and in an apparently normal and usual condition for two or three days; they could easily have been kept longer had it seemed advisable.

The tissue selected for observation was in the fins of free swimming pelagic and *Fundulus* embryos. The embryos studied were chiefly of *Fundulus* and varied from 5 to 20 mm. in total length. The most favorable subjects were from the time of hatching, 5 to 6 mm., up to 12 mm. in length. The pectoral and caudal fins were usually selected as most available for observation. In such embryos the fin consists of a central frame work formed by the jointed rays, lepidotrichia, with their attached muscles, and a superficial integument of pavement epithelium with its sub-jacent basement membrane. The finer fin rays, actinotrichia, continue the jointed rays to the margin of the fin. The fin at this stage is very thin and the epidermis lies almost in contact with the fin rays. But between adjacent rays is an interval which lodges on either side the afferent and efferent blood vessels, bordered by chromatophores, and between them a loose mass of mesenchymal connective tissue in which the cells may be readily observed.

In embryos 5 to 6 mm. long the connective tissue in the pectoral fins consists chiefly of a mass of round cells confined to the proximal portion, and beyond this mass a distal fringe or 'skirmish line' of scattered stellate cells. In the unpaired fins, which are less advanced in their development only the scattered stellate cells are represented, the invasion of the round cell mass having not yet occurred. In later stages, as in the caudal fin of the same embryo, the zone of round cells has advanced distalward among the actinotrichia nearly to the fin margin, leaving behind between the lepidotrichia an area of more mature cells, stellate and spindle, and a few fine fibers well separated by broad spaces occupied by tissue fluids. The spindle cells and fibers preponderate in the

proximal, the round cells in the distal zone of the fin. Hence, one follows the sequence of development in passing from the distal toward the proximal portion of such a fin. Older embryos show the same zones of transition but in them the formation of fibers in the proximal region is more advanced.

In mammalian tissue one finds three stages in the histogenesis of connective tissue, a primitive cellular stage, a syncytial stage, and a fibrous stage. The first is characterized by the predominance of round cells, the second by stellate, the third by spindle and lamellar cells. The same succession of cell types is present in the fins of embryo fish and there is a corresponding succession of histogenic stages. Fibers do not appear prior to the appearance of cellular processes. Fine fibers appear coincidentally with stellate cells, coarse fibers and fiber bundles develop later.

In the distal portion of the fin fine fibers first appear in the round cell area coincidentally with the transition from round to early stellate forms. At exactly this period I have observed the first indication of motion, the throwing out of pseudopods by the round cells, in the connective tissue cells of the living embryo. Fig. 1 shows such changes in two cells on the border of the round cell area near the posterior end of the ventral fin. There is at this time relatively little locomotion, as is shown in the figure by referring the position of the cells *a* and *b* to the relatively fixed point, a prominence on the margin of an adjacent chromatophore (*ch*).

The first appearance of fibers in the distal portions of the fins has been very properly connected by Harrison, and by Goodrich with the origin of the dermal fin rays from the 'scleroblast' cells which closely resemble the connective tissue cells and like them are of mesodermal origin. In the region of the actinotrichia in the distal portion of the fin, it is difficult to distinguish between the early forms of these coarse fibers and the true connective tissue fibers, but the actinotrichia are confined to the region of the last one or two joints of the jointed fin rays, and there they project, as Goodrich has shown, from between the two opposed dermal plates which form the distal section of the jointed fin ray. If therefore one studies a region proximal to the last section and

selects the interval between the jointed rays the primitive actinotrichia are thereby excluded.

In such portions of the caudal fins of 6 mm. embryos, and in equivalent places in later stages, are typical connective tissue fibers mostly occurring as coarse longitudinal bundles with fine oblique anastomoses. Single fibers occur in the intervals of the coarser bundles. It is along these fibers and fiber bundles that the stellate and spindle cells are disposed. These cells are readily seen in the living fish, though the ease of observation is subject to much variation in different individuals and to a less extent in different portions of the same embryo.

My observations were made on living embryos immersed in sea water, some with, some without the addition of chlorotone. In some cases a few drops of a saturated solution of Bismark brown were added to the sea water in which the fish was kept, the effect of which after a time was to slightly increase the color contrast between the connective tissue cells and surrounding structures. The stain seemed almost innocuous, for fish could be kept in it for several days without apparent effect on their vitality. Many of the fish thus examined were later killed, and the fins stained and mounted in toto, or sectioned. The various cell types seen in life were readily recognizable in corresponding locations in the stained preparations.

It is in life difficult or impossible to distinguish between the spindle and lamellar types, though in 'fixed' tissue they may be morphologically distinct. In the living animal one can see a stellate or a spindle cell elongate, approach and flatten itself against a connective tissue fiber or fiber bundle, becoming sometimes so attenuated as to be scarcely distinguishable from the fiber against which it lies; it may at any time acquire increased thickness. Such a relation to a connective tissue fiber is shown by the cell *b* in fig. 2. The relationship is again exhibited by the two cells shown in fig. 3, one of which *a*, approached a small fiber bundle, became flattened against it, then rotated to the opposite side of the fiber at 9.30 A.M., and later freed itself from the contact. Its locomotion can be observed in relation to the chromatophore (*ch*) which served as a fairly fixed point. Similar

cells are frequently seen flattened against the surface of fiber bundles, blood-vessels, or fin-rays, and exhibiting a slow stereotropic locomotion. Many of these cells would seem to be identical with those which in stained preparations we are accustomed to call lamellar cells.

That connective tissue cells exhibit a certain amount of motion is no new observation. It has been well known since the inflammatory reaction to injury or infection was studied in the mesentery by Arnold and others. I have observed that the extent and rapidity of the motion varies with the cell type. The round cell, or primitive type, presents relatively little motion, it being limited, so far as I have observed, to the very slow projection and retraction of minute pseudopods. Even this evidence of activity seems rather to be limited to those later phases of the cellular stage which foreshadow the transformation of the round cells to the stellate type of the succeeding stage. This transformation is indicated by the fact that the motion is more noticeable near the border of the round cell area than in its interior, and also because at the extreme margin of such a cellular area one may by careful scrutiny observe an extensive alteration from round to stellate types, some cells passing rapidly to an approximate spindle form. The type of motion exhibited by the round cells, when observable, is well shown by fig. 4, cells *a-c* being observed at the extreme margin of the round cell area, cells *d-e* just within the margin, and cells *f-g* well in the interior of the area.

While the general trend of cell change is from round to stellate to spindle cells, a change may often be observed to occur in the reverse direction, as occurred to the cell shown in fig. 5, and that in fig. 6. Such retrograde changes are less frequently observed, and the transformation is less extensive than are the progressive changes from the round to the stellate forms. The retrograde stellate phase is also more frequently of a transient character (fig. 5). Thus, a stellate cell may by retraction of its processes temporarily assume a spheroidal form but it soon again projects pseudopods and regains its stellate character. Or a typical, bipolar, spindle shaped cell may extend a third process, or even several additional processes (figs. 2, 5 and 6), but, so far as I have

observed, such processes are limited in size and usually of short duration. This reverse transformation may be likened to an elastic rebound brought about by an inherent resistance to change of form reacting against an impelling force which directs the transformation from the round to the spindle type. The cell frequently balks at the change, but the general trend from round to stellate and from stellate to spindle form is inevitable.

Motion resulting in change of form is perhaps most active in the stellate type of connective tissue cell. The general trend of this motion seems to be indicated in fig. 5 *I*, in which a typical round cell selected for observation at the margin of the round cell mass in a pectoral fin of a 6 mm. embryo was seen within a period of six minutes to elongate and then to pass through successive stellate shapes to a typical spindle form. But the succession is not always so rapid. Stellate cells exhibit all sorts of morphological transformations in rapid sequence (fig. 7) and this stage of connective tissue development is of relatively more transient duration than either the preceding or the succeeding stage. Moreover, the shape of the cell is undoubtedly influenced to some extent by its surroundings and the duration of a particular stellate, spindle or lamellar shape may in some cases be thus determined.

Likewise, spindle cells undergo considerable transformations in form, the most frequent of which undoubtedly result in the lamellar shapes on the one and in the stellate on the other hand. Because of the limitations of the microscope in the delineation of the 'third dimension' it is most difficult in the colorless living tissues to differentiate between the lamellar and spindle types of cell but the evidence of fixed and stained tissues shows the lamellar to be the more mature, the spindle the earlier type, and I have observed nothing in the living tissues to indicate the contrary unless indeed it be that both types appear to be somewhat dependent on their surroundings, for as already stated these forms, in the same cell, seem to be more or less interchangeable. That spindle cells frequently and freely revert to the stellate type there is abundant evidence. There is also evidence that these cells may be capable of still further transformations than those of mere form. A syncytial stage in the development of connective tissue has

long been assumed. That this stage in its most typical form presents those cell pictures which we are accustomed to regard as stellate cells is well known. It is generally recognized that this syncytial stage passes into one in which the fibers appear and the syncytium is replaced by a tissue of cells and fibers. The syncytial stage has been presumed to be preceded by a cellular stage and to those who have traced the origin of the mesoderm from the time of egg fertilization it would appear logical, even necessary, that at a sufficiently early period a cellular character must obtain, though Mall has questioned the preëxistence of this cellular condition. The transformation from the cellular to the syncytial condition has been ascribed on the basis of stained sections, to either of two processes: either the syncytium arises by incomplete division of preëxisting cells or the syncytium results from the fusion of the preëxisting cells. That some syncytia arise by incomplete cell division is very probably true. This appears specially obvious in such placental tissues as the superficial cells of the chorionic villi. I know of no convincing evidence that it does occur in the connective tissues.

Since I have been unable to observe mitotic figures in the living connective tissue cells of the fish which are under discussion I cannot offer any evidence pro or con the origin of a connective tissue syncytium by incomplete cell division. I have, however, frequently observed a phenomenon which simulates the fusion of processes of adjacent stellate cells after the manner of a typical connective tissue syncytium. In figs. 2 and 8 *II* the neighboring cells, which were at first entirely distinct and separate, were within a brief period seen to send out processes which on contact apparently fused. But of course one cannot say without subsequent fixation and staining of the identical cells, a process presenting the greatest difficulties, that the fusion was actual and complete. Even in stained sections the question is often difficult to determine. While the fusion was apparent I am not at all sure that it was actual. Not, however, in every case when cell came into contact with cell did such apparent fusion occur. This is shown in fig. 8 *I*, in which processes from the cells *a* and *b* came into contact tip to tip, yet though fusion seemed imminent it did not occur

and the contour of each cell at the point of contact remained clear and distinct. Moreover it would seem that since connective tissue cells move extensively along the surfaces of the syncytium that syncytium could scarcely arise by fusion of its cells.

The spindle cells exhibit a certain stereotropism. They are prone to take their position alongside a connective tissue fiber or fiber bundle or against the surface of a blood-vessel or dermal fin-ray. When in contact with a broad surface, such as that of a blood-vessel or one of the lepidotrichia, the cells frequently assume a flattened, lamellar form. This is shown by stained sections, in which that type of cell predominates in these locations, and by the observation of living spindle cells which frequently move up to a blood-vessel or a fin-ray and then become so thinned out against the surface that they finally vanish, being in the living tissue indistinguishable from the refraction lines which surround the larger bodies. Again, the spindle cells very frequently move up to a connective tissue fiber or bundle and then elongate along the narrow filament until, as before, the cell finally appears to vanish by its extreme attenuation. Such a result was observed a moment later than the recorded observation in the case of the cell shown in fig. 5I.

It frequently happens that the spindle cells after such elongation again thicken to a typical spindle form, and may even throw out other processes, but in so doing, if the cell is observed in relation to some relatively fixed point, e.g., a joint of a dermal fin-ray, a chromatophore, or a blood-vessel, it will be seen that the cell has changed its relative position; it has exhibited locomotion. Locomotion is not a distinguishing character of the spindle cell; it is exhibited by the stellate cells, possibly also to a very limited extent by those round cells which are only just beginning to present pseudopod formation. But the character of the locomotion in the several types of cells differs decidedly. In the stellate type locomotion may take any direction and resembles a very active amoeboid motion, processes being extended along the surfaces of fine fibers, then either retracted or increased in size until the whole cell has come to occupy the place of the former process. Though locomotion in the stellate cells is not entirely confined

to the direction of visible fiber lines, yet a projecting process of such a cell often appears to envelope or to become coincident with a fiber. In the spindle cells locomotion is always so far as I have observed, in the direction of the fiber lines: usually these cells merely slide along the surface of fibers, blood-vessels and similar structures.

I have observed that the stellate cells are more prone to lie in relation with the finer, the spindle cells with the coarser fibers; the coarser fibers in most cases, because of their size, being presumably fiber bundles rather than single fibers. This relationship is to be expected in as much as in stained preparations one finds the stellate cells present with those finer fibers which represent the earlier stages in fiber formation.

That fibers do lie without the cell in both embryonic and mature connective tissue is generally conceded. That they lie within the cell in reticular tissue, which in a way is comparable to an early or embryonic type of connective tissue, I have recently demonstrated by means of the Bielschowsky stain.¹ The types of fiber development by fusion of intracellular granules described by Spuler and by Lavini though perhaps not conclusively demonstrated, at least show that certain granules which are in relation with the first appearance of fibrils do lie within the substance of the stellate, mesodermal, connective tissue cells. Moreover, I have found in embryonic tissues (fig. 9) just such appearances as I have described for reticular tissue.² By means of the Bielschowsky method such appearances can be shown throughout embryonic connective tissue. I have observed them in pig embryos, of various ages, in the limb buds, the head, the cervical region, and in the back throughout the whole length of the embryo from the occiput to the caudal tip, also in the umbilical cord. In many of these locations I have made similar observations on human embryos of older stages but in which the connective tissue was still actively developing. One is at a loss to explain the method by which fibers arising within the cells arrive at a location outside the cell body when these cells are in active motion. The

¹ *Am. Jour. Anat.*, vol. 13, page 277, 1911.

² *Loc. cit.*, in which see especially fig. 4 and fig. 8, pages 285 and 289.

ectoplasmic theory of Hansen does not satisfactorily account for it and its elaboration by Mall is not as specific in this particular as one might wish. These theories do not appear to fully harmonize with the relatively active motion and locomotion of the connective tissue cells which I have observed in the fins of living fish and which Harrison, Burrows and others have also to some extent recognized in tissue cultures. The cells are not sufficiently quiescent to permit of endoplasmic retraction with deposit of ectoplasmic fibers unless this retraction is rapidly performed, in which case it should be observable in the living embryo. I have in one or two cases suspected such a method of deposit but have not as yet been able to convince myself that it actually occurs; in fact I now doubt if it occurs at all.

The ectoplasmic theory presupposes that the fibers arise at the surface of the cell. This I have found to be not always the case. The clear delineation of fibers by the Bielschowsky method makes it possible to follow their course within the cell more carefully than ever before and I find that the blackened fibrils within the cell both in pig embryos (fig. 9) and in the fish's fin very frequently pass close to the nucleus, sometimes ending almost in contact with this structure, but more frequently passing by so closely as to be in actual contact with the nuclear membrane. I am aware that Golowinski using the iron haematoxylin method, demonstrated the presence of fibers at the surface of the connective tissue cells of the umbilical cord and that the apparent relation to the nucleus was explained by him as due to obliquity of section. But I have not in my preparations been able to convince myself of the adequacy of this explanation. I have found fibers to be not always at the surface of the cell, they may and frequently do penetrate entirely through the cytoplasm of the cell, as I have previously described³ for mature reticular tissue. In the developing connective tissue, as well as in reticular tissue, such penetration of cells by the fibers is so frequent as to appear quite characteristic. It seems to me that the intimate relation of connective tissue cells and fibers in embryonic tissues can only be accounted for by tak-

³ Loc. cit., see fig. 10, page 293.

ing cognizance of the plasticity of the connective tissue cellular cytoplasm, and also of the active motion of connective tissue cells during the period in which the fibers are being formed, so that the finer connective tissue fibers become, by the cellular activity, embedded in the plastic cytoplasm of the cells during their stereotropic locomotion. The plastic character of the cellular cytoplasm is admirably shown by the rapid changes in form of the connective tissue cells in the fins of living fish embryos.

I have already stated that the spindle cells of connective tissue in the fins of living fish undergo active locomotion. In fact this seems to be a most prominent function of the spindle cell type. Most frequently the cell glides along connective tissue fibers which often appear to be thus partially enveloped by the cytoplasm. In recording this stereotropism I am able to corroborate, for the living cells of embryo fish, the observations of Harrison on tissue cultures in which he finds that the connective tissue cells are specially prone to follow along the surface of fixed objects. Such objects in normal living subjects are most frequently the connective tissue fibers and fiber-bundles already deposited, though as previously stated, I have also observed connective tissue cells moving along the surface of the dermal fin rays and of blood-vessels. In this form of activity the cells adapt themselves more or less to the shape of the surface along which they are moving. They wrap themselves about or rotate around the finer fibers (fig. 3) and they flatten themselves against the larger objects (figs. 2, 3 and 5). In this attenuated condition they still move along the surface of fibers, often at a considerable rate of speed. One such cell I have recorded in a preliminary communication⁴ was found in ten minutes to have covered a distance of 50μ , a rate of 1μ in every twelve seconds.

The striking similarity of the living, spindle, connective tissue cells to those of fixed tissue is indicated in fig. 10 which shows several such cells from a 100 mm. pig embryo. The magnification is the same as that used for the observation of the living cells. The similarity in the form of the cell and the relation of cells to fibers is apparent on comparison with the preceding figures. One

⁴ Biol. Bull., vol. 21, page 272, fig. 2, 1911.

can scarcely avoid the interpretation that the cells shown in fig. 10 were at the moment of 'fixation' moving along the surface of the fiber bundles against which they lie.

The pronounced morphological relation between the connective tissue cells and fibers cannot but have an equally close functional relation. What those functional relations may be we are not now possessed of the data to fully determine.

Further studies will be necessary to fully understand the part played by the active moving connective tissue cells in the production and growth of the collagenous fibers. So far as they are now determined the essential phases of the process in which the connective tissue cells are concerned appear to be: (1) the connective tissue arises from a primitive mass of round mesenchymal cells; (2) there is a change of form from round to stellate, to spindle, and eventually to lamellar cells; (3) certain fibers seem first to appear within the cells, possibly at their surface (Hansen, Golowinski); (4) there is formed a reticulum pervading the intercellular ground substance whose fibers may be, though they not necessarily are, identical with those first arising within the cell; (5) coincident with the origin of fibers there begin amoeboid movements in the stellate and spindle cells; (6) there is an increase in size and number of fibers in the reticulum and they aggregate into bundles, synchronously with which first the stellate and later the spindle cells move along the surface of the fiber and fiber bundles.

In conclusion I desire to express my sincere thanks to the Marine Biological Laboratory at Woods Hole, Massachusetts, for the opportunities so kindly placed at my disposal.

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PLATE 1

EXPLANATION OF FIGURES

1 From a *Fundulus* embryo, of 5.5 mm. total length, showing beginning amoeboid movements of two cells, *a* and *b*, on the border of the round cell area at the posterior extremity of the ventral median fin. The observation extends over a period of fifteen minutes. The last seven drawings were made without change of focus for the purpose of eliminating variation in form due to the examination of different levels. *a*, *b*, two connective tissue cells. *Ch*, prominence on the surface of a chromatophore, the body of the cell is not represented. The numerals in this and succeeding figures indicate the exact time at which each recorded drawing was completed.

2 Connective tissue cells, one of which, *a*, exhibits transformation from a spindle to a stellate type, and another, *b*, becomes flattened against a connective tissue fiber. There was an apparent anastomosis between cell *a* and the protoplasmic process *p* of an adjacent cell. *f*, connective tissue fiber; *j*, margin of a joint of a fin ray, giving a fixed point in relation to which locomotion may be determined. Other letters and numerals as in the preceding figures.

3 Two connective tissue cells exhibiting some locomotion. One of these, *a*, assumed a lamellar like relation to a fiber bundle while rotating about it. At 9.25 A.M. this cell became momentarily so thin as to almost escape observation. *Ch*, *Ch'*, chromatophores. Other letters and numerals as in the preceding figures.

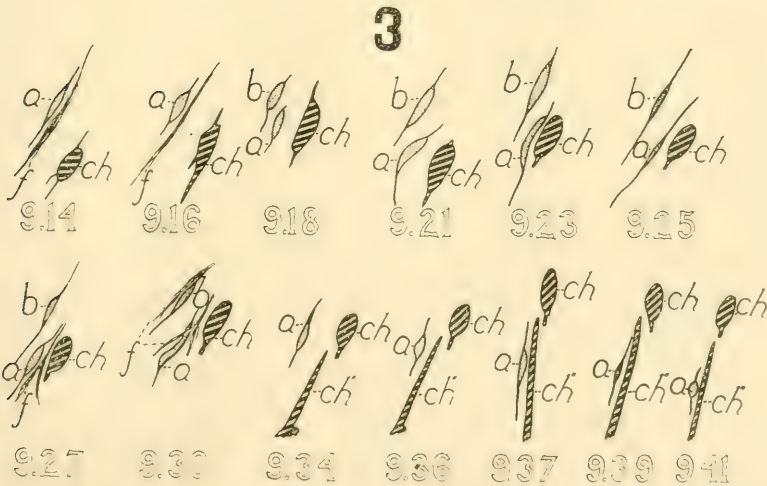
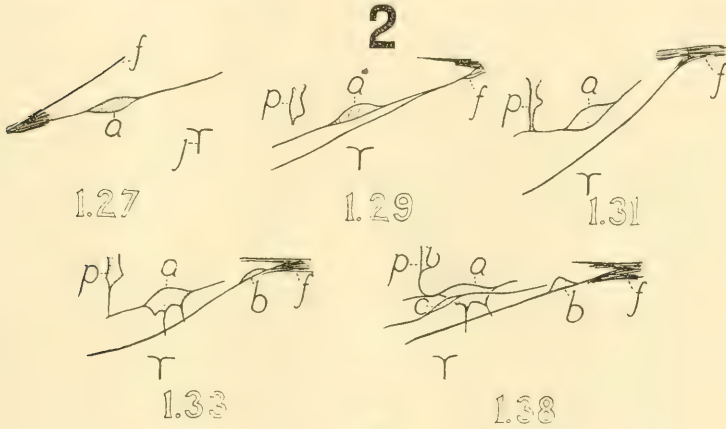
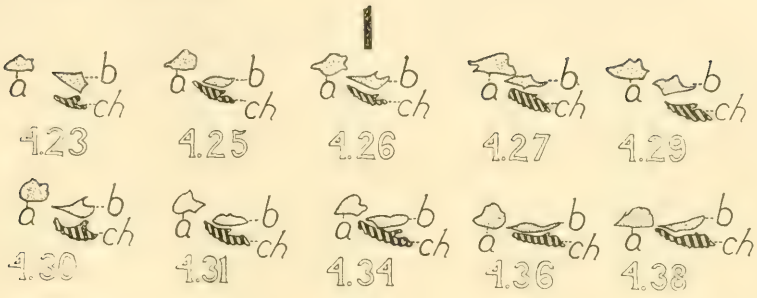


PLATE 2

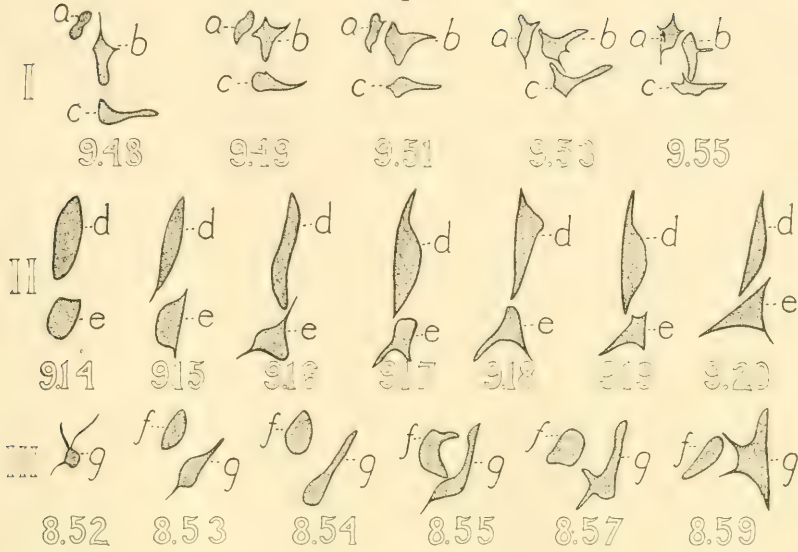
EXPLANATION OF FIGURES

4 Amoeboid motion resulting in change of form exhibited by connective tissue cells of the primitive or 'round' type. *I*, cells *a-c*, from the extreme margin; *II*, cells *d-e*, from just within the margin; and *III*, cells *f-g*, from the interior of a round cell area. *I* and *II* from the pectoral, *III* from the caudal fin. Numerals as in the preceding figures.

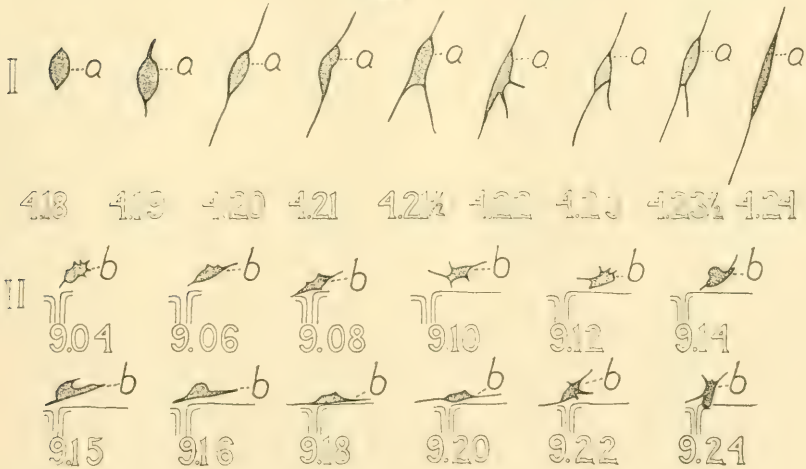
5 *I*, transformation of a round to a spindle cell in the pectoral fin of a Fundulus embryo 6 mm. long, 20 days after fertilization, 11 days after hatching. From 4.20 to 4.22 P.M. there was in this cell an apparently retrograde change from spindle to stellate form but at 4.24 P.M. this had been proven temporary. *II*, transition of a stellate cell to a temporary spindle form. Letters and numerals as in preceding figures.

6 Apparent retrograde change from spindle to a stellate form in a cell undergoing rather slow locomotion. The stellate phase of such cells is nearly always temporary. From the same embryo as fig. 5; *cap.*, blood-capillary. Other letters and numerals as in the preceding figures.

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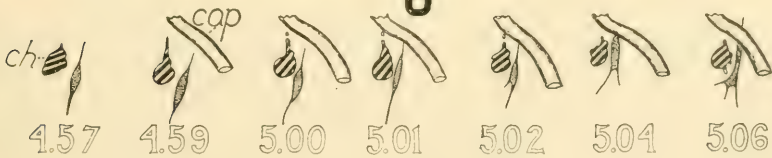


PLATE 3

EXPLANATION OF FIGURE

7 Stellate connective tissue cells from the fins of four embryo fish, *I-IV*, exhibiting rapid change of form. Owing to difficulties of observation it is not possible to make drawings oftener than at 1 to 3 minute intervals; hence, the actual changes of form were much more frequent than the record shows. *A-K*, nine connective tissue cells. Numerals as in the preceding figures.

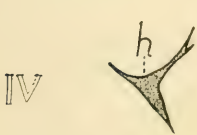
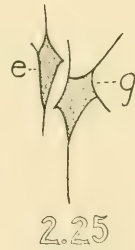
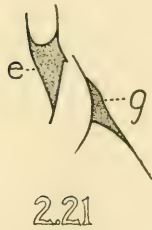
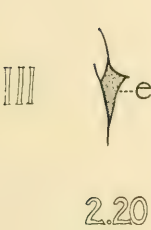
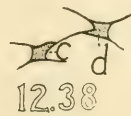
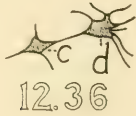
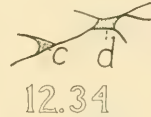
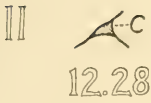
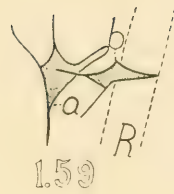
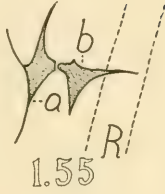
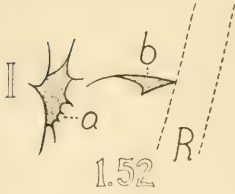


PLATE 4

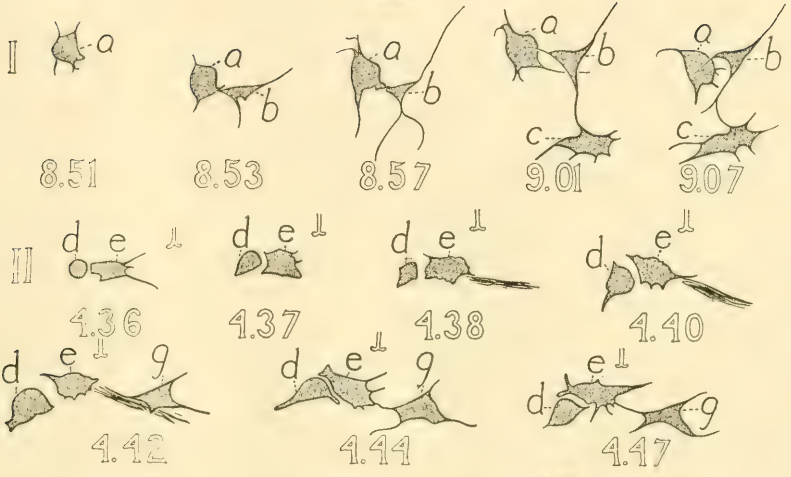
EXPLANATION OF FIGURES

8 Stellate connective tissue cells exhibiting locomotion and, on contact, apparent fusion. *I*, from the pectoral fin of a 10 mm. *Fundulus* embryo. The fusion between cells *a* and *b* is apparent only. *b* and *c* appear to form part of the anastomosing syncytium. *II*, from the caudal fin of a 6 mm. *Fundulus* embryo. Cells *e* and *g* on coming into contact at 4.44 p.m. apparently fused after the manner of the cells which form the delicate early connective tissue syncytium. Letters and numerals as in the preceding figures.

9 Stellate connective tissue cells in the subectodermal mesenchymal syncytium of a 25 mm. embryo pig. Fibrils pass through the cells very close to the nucleus. Bielschowsky stain.

10 Connective tissue cells from the praevertebral (*I*) and intermuscular (*II*) connective tissue of a 100 mm. pig embryo. The form of the cells and their contact relations to adjacent connective tissue and muscle fibers is strikingly similar to the amoeboid connective tissue cells of the living fish embryo. The appearance suggests that such cells were quite probably moving along the surfaces with which they are in contact.

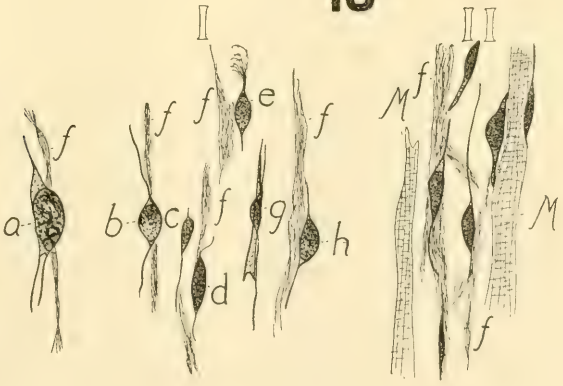
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A COMPARATIVE MICROSCOPIC STUDY OF THE INTERCALATED DISCS OF VERTEBRATE HEART MUSCLE

H. E. JORDAN AND K. B. STEELE

From the Anatomical Laboratory of the University of Virginia

TWENTY-THREE FIGURES

CONTENTS

I. Introductory.....	151
II. Material and methods.....	152
III. Descriptive.....	153
A. Adult material.....	153
1. Monkey.....	153
2. Bat.....	157
3. Guinea-pig.....	159
4. Chipmunk.....	160
5. Opossum.....	162
6. Humming-bird.....	162
7. Turtle.....	162
8. Toad.....	162
9. Frog.....	163
10. Trout.....	164
B. Young and foetal material.....	165
1. Guinea-pig.....	165
a. Second, third and fourth weeks.....	165
b. First week.....	165
c. Last week of gestation.....	165
2. Cat of four days.....	165
3. Child of four years.....	165
IV. Discussion.....	167
V. Summary and conclusions.....	172
Literature cited.....	173

I. INTRODUCTORY

On the basis of histologic findings in the heart of the humming-bird, Jordan ('11) believes he has demonstrated that intercalated discs are not correctly interpreted as intercellular elements marking cell boundaries, as recently again urged in the papers by

Zimmermann ('10) and his students, Paleczewska ('10) and Werner ('10). It is the purpose, in part, of this investigation to present further facts in support of the non-cellular interpretation of vertebrate heart muscle.

Marceau ('04) states that he was unable to demonstrate discs in the heart muscle of vertebrates lower than birds;¹ also in goose and duck. Moreover, he says that discs develop only some time after birth. The further object of our research is to test the factual basis of these assertions and to determine, if possible, from structural marks, the probable function of the intercalated discs.

II. MATERIAL AND METHODS

The material studied includes heart muscle of adult human, monkey, sheep, bat, cat, guinea-pig, mouse, rabbit, chipmunk, opossum, humming-bird, lizard, turtle, toad, frog and trout; also of guinea-pig of last week of gestation, and of first, second, third and fourth weeks of post-natal life; of a cat embryo of four days, and of a four-year-old child; and of *Limulus*.²

Zimmermann's technic³ was employed except for adult human, cat, rabbit, sheep and lizard hearts. Mammalian cardiac muscle of the latter group has been fully described and illustrated by Werner. We shall confine our descriptions for the most part to the heart muscle of forms not previously studied. The descriptions in every instance apply to the ventricular tissue.

¹ In the second volume (1911) of 'Plasma und Zelle,' Heidenhain says 'Schaltstücke wurden bisher in keinen Falle bei niederen Wirbeltiere beobachtet; sie finden sich by Vögeln zum ersten Male.'

² We are indebted to Messrs. H. F. Jackson and E. L. Powers for kindly collecting the material of toad, frog, trout and *Limulus* at Cold Spring Harbor, L. I.; and to Dr. W. H. Schultz, of the Hygienic Laboratory, Washington, for the young and foetal guinea-pig hearts.

³ Following this technic, small pieces of tissue were treated for twenty-four hours with a solution of 90 parts of absolute alcohol plus 10 parts of 25 per cent HNO_3 . The tissue was then washed in several changes of 94 per cent alcohol or until the latter remained neutral to litmus paper. It was then passed into distilled water, from which it was transferred to a solution of 1 gram of Grüber's haemalum in 10 cc. of water. Here it remained for from eight to ten days when it was washed in distilled water and then carried through the ordinary steps of the paraffin technic. In our own experience we have obtained equally successful preparations by staining sections for twenty-four hours on the slide.

III. DESCRIPTIVE

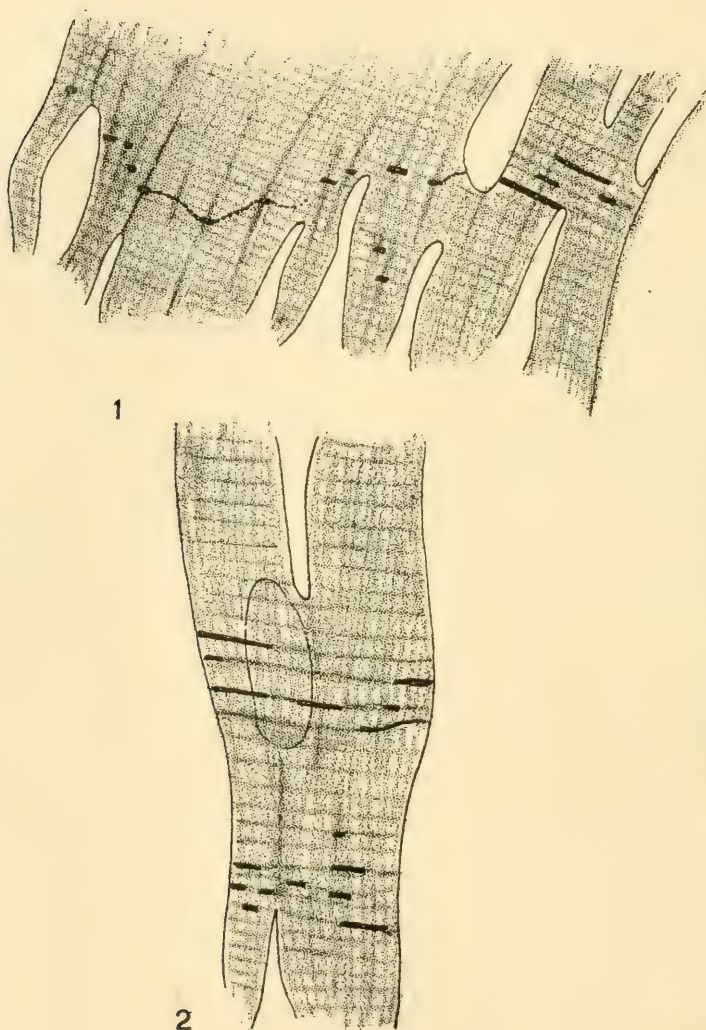
A. ADULT MATERIAL

In every case the myocardium consists of a close-meshed network of coarser and finer branching striped muscle trabeculae.

1. Monkey

When a large expanse of tissue is under view, the discs are seen to lie more or less closely aggregated in definite regions. There is evidently roughly an alternation of disc-containing and disc-free areas (figs. 1 and 2). On close examination the disc-containing areas for the most part correspond to the axial regions of the mesh. In physical terms these regions correspond to areas of greatest stress during contraction (figs. 1 and 2). The discs appear commonly in three distinct forms: (1) they may be compact and wide,⁴ i.e., as wide as a single fiber; (2) they may be narrow, i.e., not much wider than a single fibril; (3) they may consist of rows of spherical granules, connected severally by a delicate deep-staining membrane. These three main types of discs are all represented in fig. 1. The 'compact' discs under higher magnification are seen to be composed of rows of longer or shorter rod-like granules. The rows of spherical granules usually span the intervals between adjacent discs of more compact forms (forms 1 and 2). The myofibrillae are clearly evident, singly and in bundles, giving the fiber a distinct longitudinal striation. The striations (fibrillae) passing through the smaller discs of form 2 are much coarser than the adjacent fibrils (fig. 1). These fibrils are evidently modified, being probably in a state of greater contraction; and the narrower discs would seem to be foci of still greater contraction in the fibrils. If this interpretation is valid—as many facts about to be detailed very strongly indicate—then the wider discs are reasonably conceived as the result of a fusion or association of narrower discs; and the

⁴ 'Wide' and 'narrow' refer to the transverse axis of longitudinal sections, 'coarse' or 'robust' and 'delicate' to the longitudinal axis, and 'deep' and 'superficial' to the diameter of the uncut fiber.



All figures unless otherwise specified are magnified 1800 diameters.

Fig. 1 Expanse of cardiac muscle of monkey in longitudinal section, showing several types of intercalated discs, their general disposition in the axis of the mesh; and their relation to each other, the dark bands, and the myofibrillae.

Fig. 2 Longitudinal section of two medially fused muscle trabeculae of monkey heart, showing the axial disposition of the discs, and the super-nuclear location of several.

rows of granules as areas of local contraction in otherwise relaxed and distinct fibrils, the inter-granular membrane possibly representing a condensed membrane of Heidenhain (*M* line; mesophragma, Heidenhain).

The discs are almost invariably at the level of, and, where present, displace the dark (anisotropic) bands.⁵ However, they are frequently wider than these bands. Occasionally, when robust, they may extend approximately halfway or even entirely, through the lighter band (fig. 6). But they are certainly not generally bounded on both sides by Krause's membrane (telophragma, Heidenhain), an observation urged in favor of an intercellular interpretation, as has been stated by some investigators, e.g., Heidenhain.

In fig. 2 all of the discs appear as wider or narrower more or less compact granular bands. The two separate collections are localized at the points of stress. One disc appears to span the line of junction. Several lie partially over the nucleus. All are superficial and of comparatively little depth.⁶ These structural variations, superficial and occasional super-nuclear position, render untenable interpretation as cement lines. Fig. 3 illustrates four successive superficial discs unconnected by 'risers' to form 'steps.' Moreover, they gradually shade laterally into the anisotropic bands which they in part displace. In fig. 4, three discs are shown overlying the deeper nucleus. In fig. 5 are illustrated two wide discs of finely granular character. Such discs are fairly common. In this particular fiber the two discs lie at slightly different depths. The important point is that in passing from a

⁵ The term anisotropic is not here employed in a definitely physical sense. It is used simply to denote the darker-staining substance in the *Q* and *Z* band of striped muscle, in accordance with common custom. Careful study of cardiac muscle with the micropolariscope, under the same physical conditions that very strikingly revealed anisotropic vegetable fibers and inorganic crystals, failed, however, to disclose any definitely anisotropic substance in the myofibrillae. The point urged is the similarity between the darker transverse and the intercalated discs. It would seem, moreover, that not all striped muscle can be resolved into sharply defined isotropic and anisotropic discs. On the other hand, as far as the micropolariscope gives evidence, 'stripes' appear in the absence of anisotropic granules.

⁶ Hence 'discs' strictly defined is a misnomer; they are more properly designated 'bands.' The *Q* and *J* 'bands,' moreover, are more correctly denominated 'discs.'

higher to a lower level of focus, when one disc fades out of view as the other passes in, no differentiated connecting substance ('riser') appears, as one would expect if these two discs represented portions of an irregular cement line cut tangentially, as interpreted by Zimmermann and his students.

The most plausible interpretation, it would seem, must regard the dark-staining granules or rodlets of the 'discs' as local modifications or contractions at anisotropic levels of the myofibrillae.

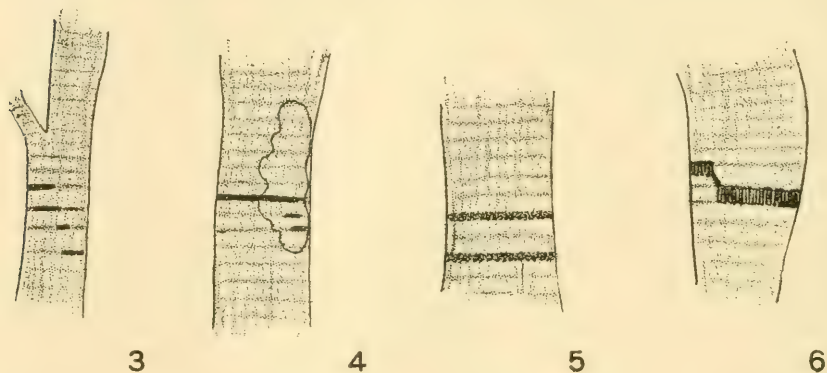


Fig. 3 Branching fiber of monkey heart with four plate-like discs, showing their relation to the dark bands.

Fig. 4 Short portion of fiber of monkey heart showing super-nuclear position of three discs.

Fig. 5 Two granular discs of monkey heart at different levels of focus. In passing from the higher to the lower level of focus no connecting membrane or 'riser' appears.

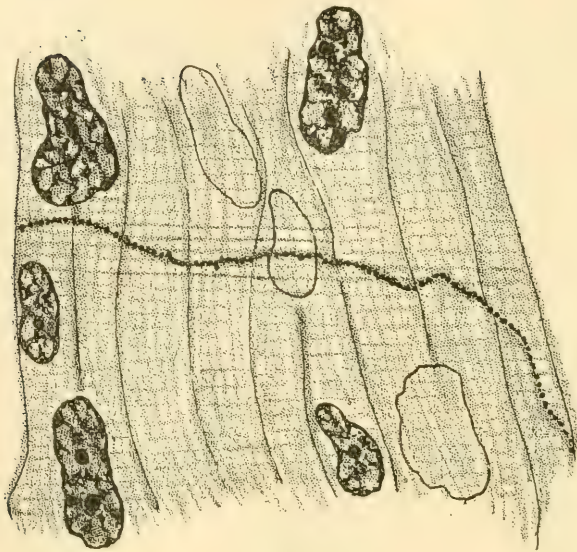
Fig. 6 A two-step, comb-like, disc of monkey heart, in longitudinal width equal to that of the isotropic and two anisotropic discs. The two portions are connected by a coarse deep-staining membrane. The 'teeth' of the 'comb' are interpreted as locally contracted portions of adjacent fibrillae.

Fig. 6 shows a rare variation. Here the disc may extend the longitudinal distance of half or even an entire lighter band. In form it has a 'comb' structure. When at different levels, as here, the several sections are connected by a robust deeply-staining membrane. The appearance simulates protoplasmic processes or 'intercellular bridges.' However, if this were a correct interpretation, they would undoubtedly be very much more numerous.

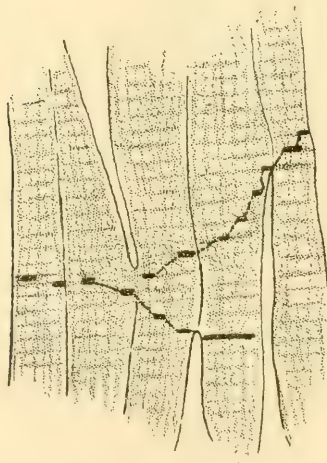
The 'teeth' of the 'comb,' we believe, must be interpreted as local, elongate contractions of the myofibrillae. Occasionally such discs consist of only one or several 'teeth;' again three or four 'combs' may appear in longitudinal series, giving the appearance of a contracted striped insect muscle. Frequently successive discs limit a region of darker sarcoplasm; frequently also discs lie in regions of darker sarcoplasm. The significance of these observations is that they indicate a relationship between discs and a condition of contraction. Possibly the discs outline limits of special physiological states. The loose granular discs are perhaps simply 'comb'-discs with shorter 'teeth.'

2. *Bat*

In the heart of the bat, in addition to the types of discs described for monkey, there appear discs in the form of a line of dark-staining, connected, spherical granules extending across as many as eight separate fibers; and directly over underlying nuclei (fig. 7). Such a structure does not even vaguely suggest a cell membrane or cement line. It becomes intelligible only in terms of local modifications (contractions?) at approximately the same level in many adjacent fibers. It would seem to represent the morphologic picture of a physiologic state sharply localized longitudinally, widespread laterally. In fig. 8 are shown two series of narrow discs connected by more or less delicate granular inter-disc membranes ('risers'). These inter-connections, and the localization of the discs in the axial region of the muscular mesh, here again suggest the effect, or phase, of a definite physiologic state. The inter-disc membrane may represent less extreme local contractions in the intervening fibrillae, or a dislocated modified membrane of Heidenhain, or possibly simply 'anisotropic' granules obliquely aligned. Careful focussing reveals the fact that these complex step-like discs lie superficially throughout and encircle the fiber in ring-fashion. This single observation disposes definitely of a cell-border interpretation.



7



8

Fig. 7 Longitudinal section of cardiac muscle of bat, showing a granular intercalated 'disc' running across eight fibers.

Fig. 8 Heart muscle of bat showing two long step-like discs winding superficially around their fibers. The 'risers' probably represent anisotropic granules aligned longitudinally, or perhaps a distorted membrane of Heidenhain.

3. Guinea-pig

Fig. 9 illustrates the typical condition in adult guinea-pig. The discs are again aggregated in more or less definite areas; they may be wide or narrow, delicate or robust, compact or loosely granular. At the left, two discs are shown connected by a row of granules; a similar disc, of mono-serial coarse granules, extends uninterruptedly across the two middle fibers. The same condition, producing the disc-structure, prevails at approxi-

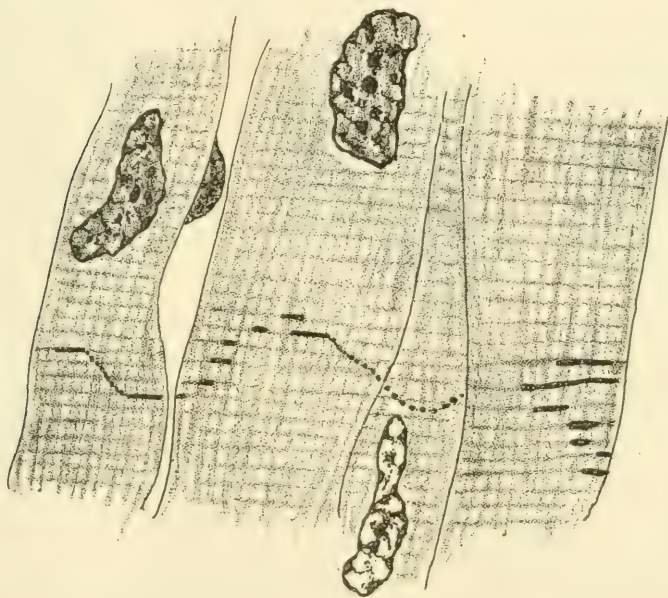


Fig. 9 Section of guinea-pig heart muscle illustrating the several types of discs, and their relation to each other, the fibrillae and the darker bands.

mately the same level in the four adjacent fibers; the appearance is incompatible with a cement line. Fig. 10 shows a disc of three 'steps' and two 'risers' extending across a nucleus. Fig. 11 illustrates a patch of compact and pale granular discs. Fig. 12 shows a variation occasionally met with in the guinea-pig and other mammals. Such a disc has not the remotest resemblance to a cement line or to an intercellular bridge. The thickenings

(blocks). of the discs are most likely local contractions in the fibrils, the connecting deeply-staining 'membrane' being formed of the lateral coalescence of less extreme locally contracted portions of intervening fibrils; or it represents perhaps a thickened, distorted membrane of Heidenhain; or it may be the product of the combination of both of these elements. This is a more common type of disc in young hearts.

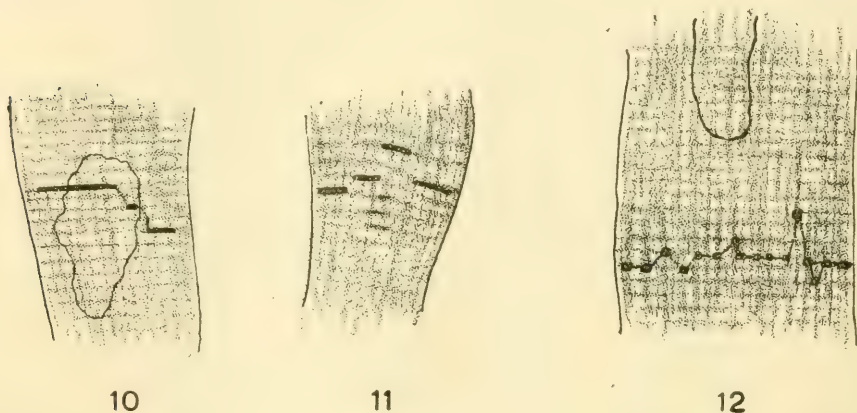


Fig. 10 Guinea-pig heart muscle fiber, showing the super-nuclear position of a three-step disc.

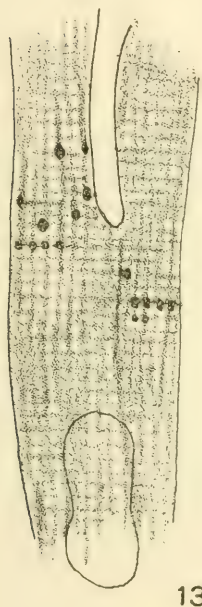
Fig. 11 Guinea-pig heart-muscle fiber, showing compact and granular varieties of plate-like discs. The loosely granular discs are the less highly developed type.

Fig. 12 Guinea-pig cardiac fiber, with block-like discs at different anisotropic levels and connected by delicate granular membranes giving a zig-zag appearance to the complete structure. The 'blocks' are locally contracted portions of the fibrillae, the connecting membranes represent probably 'anisotropic' granules in linear arrangement, i. e., M or Z.

4. *Chipmunk*

Heart muscle of the chipmunk exhibits all the types of discs above described. It shows also especially clearly and abundantly a type illustrated in fig. 13. Here the discs appear as oval thickenings on the fibrils. In other locations three or four (still more elongated) may appear at successive anisotropic levels, giving the appearance of deeply-stained striped insect muscle. Fig. 14

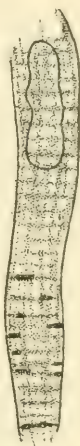
shows a step-like disc with six 'steps' and five 'risers.' The 'steps' consist of deep-staining granules; the 'risers' are more delicate granular structures. Several of the discs shade off laterally beyond the 'risers,' into more compact granular anisotropic bands. It would be impossible, we believe, to interpret this structure in terms of a cement line or cell boundary. The 'steps'



13



14



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Fig. 13 Branching cardiac fiber of chipmunk, with numerous isolated larger and smaller oval discs, for the most part at the darker levels of the fibrillae.

Fig. 14 Cardiac fiber of chipmunk with step-like discs, composed of coarse granular thick 'steps' and delicate granular 'risers.'

Fig. 15 Fiber of humming-bird heart, showing the short, compact deeply-staining discs characteristic of this muscle.

of the disc here are clearly at the levels, and displace portions, of the darker bands, the entire structure lying superficially.

5. *Opossum*

Conditions in the heart of the opossum are almost identical with those in guinea-pig. As concerns the intercalated discs, they differ only from their homologues in guinea-pig in being somewhat paler, more delicate and apparently less numerous. The deeper-staining character of the disc-containing portion of the muscle trabeculae is especially conspicuous in the opossum heart.

6. *Humming-bird*

Fig. 15, added chiefly for the purpose of completing the series of illustrations, shows a fiber of humming-bird heart muscle to illustrate the relative abundance, comparative form, and typical appearance of this tissue. Humming-bird heart muscle has been fully described in a former paper⁷ by one of the authors.

7. *Turtle*

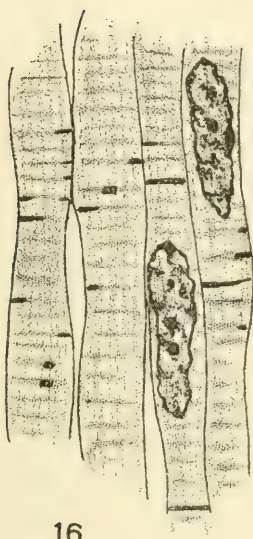
In turtle heart the discs are fairly abundant (fig. 16). The discs here all appear as narrower or wider plates. They are again superficial and at the anisotropic levels, displacing the darker bands, and in many instances shading gradually into them at one or both ends. None of the rare varieties above described for mammalian muscle appear in turtle nor in lower forms, even step-like discs being very rare. The localization of the discs in definite transverse areas is apparently absent. The discs here are stouter than in frog and toad.

8. *Toad*

Fig. 17 illustrates conditions in the heart muscle of toad. The majority of discs are very narrow, though occasionally discs the width of an entire fiber appear. The discs are situated super-

⁷In this paper the darker band was regarded as the Q band without consideration of the possibility that it may represent the Z line modified in contraction. This possibility is discussed below.

ficially and are of little depth, and apparently promiscuously placed. On close examination, under higher powers, the apparently compact delicate discs are seen to be granular. They are abundant only in certain areas, and not generally as plentiful as this particular illustration would indicate.



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Fig. 16 Four adjacent fibers of turtle heart. The discs are superficial, numerous, and very like those of bird heart; always at anisotropic levels, usually compact and only very rarely in 'steps.'

Fig. 17 Cardiac muscle of toad.

9. *Frog*

Practically the same description holds for frog (fig. 18) as for toad. The important point is the presence of discs in tissue below birds, where they have been denied. There appears absolutely no evidence here that they mark cell boundaries.

10. Trout

In heart muscle of trout (fig. 19, representing several levels of focus), the discs are distinctly fewer in number than in higher groups. Occasionally also the oval type appears as thickenings

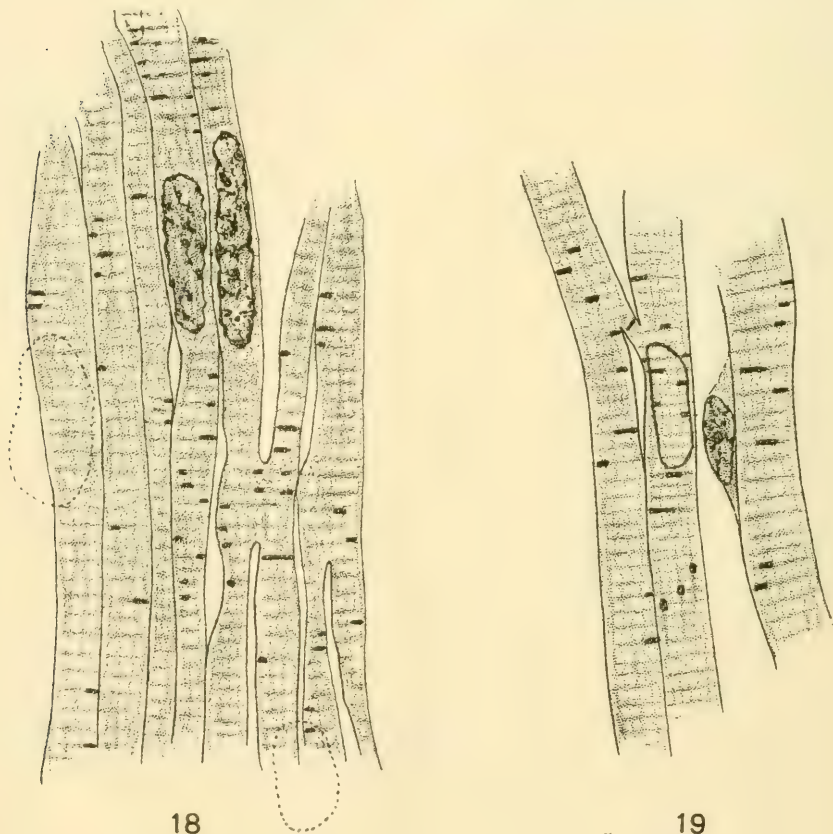


Fig. 18 Cardiac muscle of frog.

Fig. 19 Cardiac muscle of trout. The intercalated discs are similar to the simpler types of hearts of higher forms, but distinctly less numerous.

on the more distinct stouter (contracted) fibrils. The relation with respect to the nuclei and the anisotropic bands is the same as described for higher vertebrates. The discs are more abundant in contracted (darker) regions, and near branches. Occasionally

they extend across two fibers, and very frequently the discs are at the same level in adjacent fibers. They are always superficial, never appearing in the mid-line of a fiber when the nucleus is in focus. They appear in patches, and without regard to the position of the nuclei, and are only very rarely in 'steps.'

B. YOUNG AND FOETAL MATERIAL

1. Guinea-pig

a. Second, third and fourth weeks. In young guinea-pigs of the second to fourth week intercalated discs are already present (figs. 20 and 21). The majority, however, are in the shape of very narrow bands. These may be arranged in 'steps' connected by 'risers.' Many of the apparently compact discs are resolved under higher magnification into a series of blocks (local thickenings of fibrils), and the discs in general are less compact than at older stages. Again there is absolutely no evidence of cells and boundaries.

b. First week. During the first week discs are already present, and at the usual levels, but all have a light-staining character. In this tissue the granular discs shade gradually laterally into the darker bands.

c. Last week of gestation. During the last week of gestation, coincident with the appearance of striations, discs first make their appearance (fig. 23). No indication of discs could be seen at any earlier period. The discs here are narrow and consist of faintly-staining granules.

2. Cat of four days

In a cat embryo of four days the discs are already sparsely present. They are evidently just making their appearance. They are narrow, pale and granular.

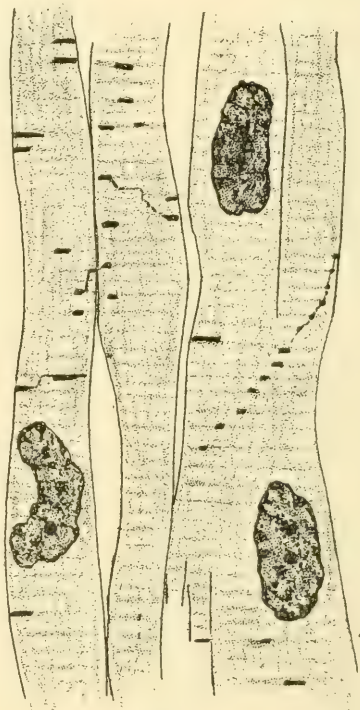
3. Child of four years

In this tissue the discs are abundant, and mostly of the type of narrow plates of slight depth. Relative to their number in the

adult heart, however, they are meager in amount. Whether this fact is due exclusively to the young, or, in part also, to the diseased condition (tubercular meningitis) is not at present clear. In the case of guinea-pig it is certain that the discs are relatively



20



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Fig. 20 Cardiac muscle of guinea-pig of four weeks of post-natal life. The intercalated discs appear aggregated in the axis of the mesh, are of simple character and mostly of paler or darker granular composition.

Fig. 21 From same preparation as fig. 20, showing the beginning of the formation of step-like discs.

less abundant and simpler in young than in the adults. Possibly the same relation obtains throughout mammals. However, there may also be a relationship between the discs and the condition of health and disease, a point which is now being investigated.

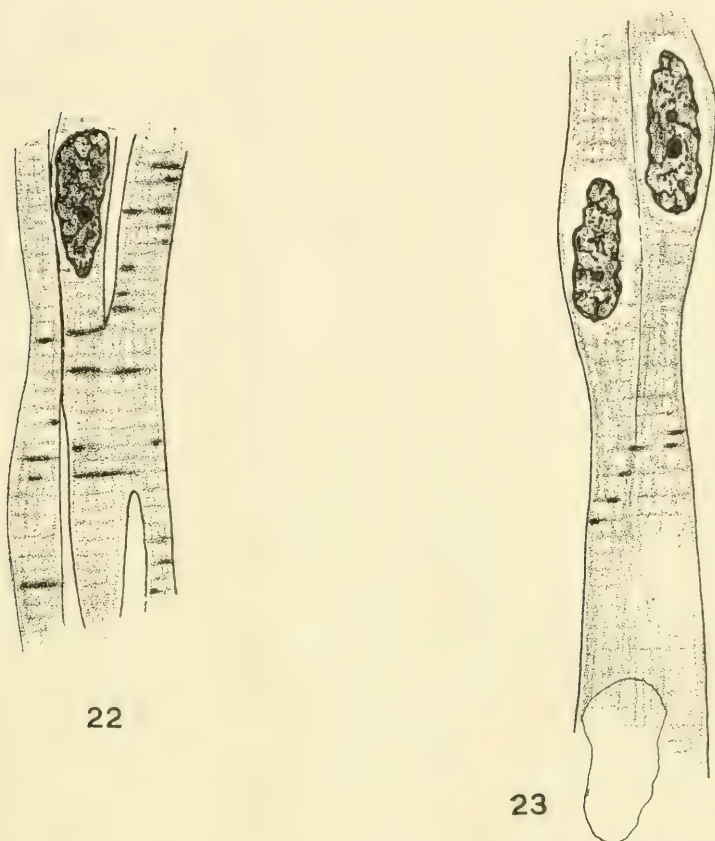


Fig. 22 Cardiac muscle of guinea-pig of first week of post-natal life. The discs are abundant, but exclusively of the granular (or less compact) narrow disc type.

Fig. 23 Cardiac muscle of guinea-pig of last week of gestation. Cross striations and discs are just beginning to make their appearance.

IV. DISCUSSION

An attempt to establish a relationship between rate of heart-beat and the number of discs was unsuccessful. If a series of animals, ranged according to the reported and observed relative abundance of intercalated discs, be compared with the same series ranged according to the rate of heart-beat, a correspondence appears at certain points between as many as three successive

members (e.g., dog, man, sheep). But at other points there is absolutely no correspondence (e.g., sheep, cat, rabbit). The relationship, therefore, if it exists at all, cannot be a simple one. Factors, besides *rate* of beat, must affect the relative abundance of the discs. Such factors may be the *force* of the beat, or the instant or total *amount of work done*. That the rate, simply, does not determine the number of the discs appears furthermore from the fact that the discs are more abundant in the adult than in the young (e.g., guinea-pig and man), whereas the rate of heart-beat relative to age varies in the reverse ratio.

Moreover, the number of the discs varies in different portions of the same heart and in different individuals of the same species. This may mean, however, that they vary according to the phase or state of function, or perhaps according to the total amount of function (i.e., age of the individual). The observations regarding the relative abundance of the discs above stated may thus have no absolute (final) significance. Sufficient observations under uniform conditions have not yet been made for an accurate seriation of heart muscles from the standpoint of the abundance of discs. The physiologic significance (normal and abnormal) of these structures will appear in full only after a careful comparative study of the same individual under varying internal and external states, both normal and morbid; of animals of the same species at different ages; and of animals from the various groups under relatively uniform conditions of age, health and function. When all the factors helping to determine the presence and abundance of intercalated discs are thus known and accounted for, it may become possible to arrange animals in identical series from the standpoint both of the rate of the heart-beat, and from the number of the intercalated discs. It seems clear that a relationship of some degree exists between the presence and abundance of these discs and function (rhythmic contraction), but in detail the relationship remains obscure.

In a histologic study of the lung of the white mouse one of us (Jordan) recently discovered that the tunica media of the proximal end of the pulmonary arteries consists of striped (cardiac) muscle for a considerable distance. This seemed to offer, there-

fore, an excellent material for testing Heidenhain's interpretation of the intercalated discs as regions where new sarcomeres ('inokommata') are being added to the growing cardiac fibers. According to Heidenhain, the heart can enlarge only by interstitial growth, i.e., by terminal additions to the cardiac elements (trabeculae). In the case of the pulmonary arteries, however, there appears no reason for postulating growth by this method; the media here, developed from truncus arteriosus to be sure, *can* nevertheless, undoubtedly increase in amount in the same way as elsewhere in arteries. Moreover, striated muscle elsewhere does not increase by means, nor show evidence of, intercalated discs. But intercalated discs are present in the pulmonary media; furthermore the greater abundance of the discs here coincides with the time of less rapid growth, and less close developmental relationship with the heart. The presence of intercalated discs in the media of the cardiac end of the pulmonary arteries in the mouse would seem to be correlated with the 'beat' (strain?) here occurring in common with the heart. Still other facts controverting Heidenhain's interpretation that the intercalated discs provide for the 'interkalare Längenwachstum' of the cardiac fibers are: (1) the absence of transition stages between the discs and fully formed sarcomeres; (2) their absence during stages of most rapid (foetal) growth; (3) their numerical increase even after the heart has attained its normal bulk; (4) their presence in aged and diseased hearts; and (5) their considerable structural variation—every type capable of resolution, however, into very similar elementary units.

Militating most strongly against Zimmermann's interpretation of the discs in terms of intercellular elements, is our observation of the superficial location of the complex step-like forms. The more complex step-like types appear only where the entire fiber is included within the plane of section. Under such circumstances the successive 'steps' can be traced completely around a fiber by lowering and again raising the level of focus. Many such are then seen to form rings or even short spirals. The discs are of course not complete in the step forms, but are interrupted, con-

sisting of 'steps' at different anisotropic levels connected by delicate membranes spanning the intervening 'isotropic' bands.

Attempts to alter the number of the discs experimentally by stimulation with varying strengths of an electric current have proved unsuccessful. Nor are they appreciably affected in tissue fixed in a state of rigor mortis. Material is now being collected for a study of these discs in various pathological conditions of the heart. Discs could not be demonstrated in *Limulus* heart.

A comparison of the illustrations accompanying the articles by Zimmermann's students, Werner and Palczewska, shows that in the human heart the discs are commonly bounded on both sides by the so-called 'Krause's Z-lines,' whereas in lower mammals the discs are narrower than the space between two Z-lines, and consequently bounded on only one side by this line. Heidenhain likewise illustrates the discs in human heart muscle as bounded on both sides by a Z-line. Granting that this interpretation of the striped condition is correct, especially then in man do the discs correspond to the *Q* or reputed anisotropic levels—as we have urged on the basis of a different interpretation—and in so far support our contention that they represent modifications of the fibrillae at anisotropic levels. But all of these illustrations differ from the far more widely prevalent condition of our material, in that the so-called 'Z-line' or 'Krause's membrane' is represented much too delicate. The darker stripe (seen both in fresh and stained material) is usually stout, and frequently almost half as wide as the alternate lighter segments; this is more particularly the case in human material. Since the sarcolemma is only occasionally, and then only imperfectly, festooned between these lines no definite suggestion is given of a 'Krause's membrane.' Especially in the regions where the discs appear abundantly are the dark stripes robust. Having naturally directed our attention chiefly to these regions, we interpreted appearances as indicating a condition of semi-contraction, according to the illustration of Tourneux (see *Traité d'Histologie*; par Prenant, Bouin et Maillard; tome 1, p. 442. Paris).

It seems possible, however, in the light of this illustration and the theoretical interpretation involved, that the fibers are in

condition of full, or nearly full, contraction. During contraction the substances of the anisotropic and isotropic bands are supposed to intermingle and ultimately change their relative locations. Such a transition condition may account for the indistinct, or absence of, stratification of the anisotropic and isotropic substances under the micropolariscope. Moreover, in the contracted condition (according to Tourneux' diagram; see also M. Heidenhain, 'Plasma und Zelle,' '11, p. 677) the darker stripe is at the level of the Z-line, itself supposed to consist of anisotropic substance. The Z-line seems to have thickened by reason of the accumulation of 'anisotropic' substance about it, forming the 'contraction band' of Rollet. Thus the darker stripe may indeed represent the Z-line, plus considerable additional anisotropic substance. The 'Z-lines' of Heidenhain and Zimmermann correspond apparently to the darker lines in our specimens, representing more likely a 'contraction band.' The illustrations of these investigators are faulty in that they show the darker stripes too delicate, always single, continuous, and too uniform. If the darker stripes are indeed the Z-lines, now grown robust in contraction, the regions of the fibers containing the intercalated discs are in a state of more pronounced contraction, according to the theory of Rollet and Tourneux. This deduction, then, is in complete accord with our position that the discs are somehow a concomitant of contraction; and further that they represent modifications (irreversible contractions?) of the fibrils at the dark (anisotropic) levels, the anisotropic substance having shifted in contraction to the Z-line. It seems clear that a complete elucidation of the question of the structure and function of the intercalated discs awaits fuller knowledge of the physical and chemical changes undergone by the cardiac myofibrillae during contraction. Our interpretation of the robust, sometimes double, dark stripes (the only stripes visible in non-human material) as the anisotropic bands seems in closer accord with our knowledge of skeletal and striped muscle generally.

V. SUMMARY AND CONCLUSIONS

1. By the use of Zimmermann's technic it was possible to demonstrate intercalated discs in all heart muscle examined, except that of *Limulus*. Of lower vertebrates the material included that of turtle, lizard, frog, toad and trout, in which forms the presence of discs has been denied.

2. In guinea-pig, in which form only the matter was tested, intercalated discs appear early during the last week of gestation, coincidently with the appearance of striations. A progressive increase in number, complexity and density was noted during the first, second, third and fourth weeks of post-natal life. A similar more pronounced difference obtains between the heart of the young and that of the adult guinea-pig. In a cat embryo of four days the discs are already present but few in number, pale, and loosely granular in structure.

3. Compared with mammals (e.g., monkey, bat, chipmunk), in lower vertebrates the discs become progressively less numerous (except in birds, e.g., humming-bird), narrower, less compact (more granular) and less complex. Conditions with respect to the discs in young mammalian hearts are very similar to those in the hearts of lower vertebrates. With increase of age, there is a progressive increase in number, complexity, density and width of discs (e.g., guinea-pig).

4. The comparative study of vertebrate heart muscle gives no evidence favoring the interpretation of the discs as structures marking cell boundaries, e.g., cement lines or intercellular bridges.

5. Specific points in the evidence against an intercellular interpretation are: (a) their superficial location; (b) their relationship to the dark ('anisotropic') band, i.e., they displace these bands and shade laterally into them; (c) their position frequently over a nucleus; (d) their relation to the myofibrillae; (e) their random arrangement with respect to the nuclei; (f) structurally and tinctorially they seem to be of the same nature as the so-called anisotropic substance; and (g) their absence before the appearance of striations.

6. The discs are interpreted in terms of local contractions (or aggregations of 'anisotropic' granules) in the muscle fibrils. The different modes of association of such single contraction foci give rise to all the various types of discs described, i.e., granules, blocks, ovals, plates (composed of closely apposed longer and shorter rodlets), 'combs,' 'steps' and saw-teeth forms.

7. The presence of discs would seem to be correlated with the function of rhythmic contraction characteristic of cardiac muscle, and may represent a fixed phase of a contraction wave (local or general), or more probably is the result (of the nature of an irreversible strain condition) of the total amount of function. The latter idea is supported by the fact of (a) their absence in the mammalian foetus, and their increasing abundance and coarseness with age; (b) their general location in lines corresponding roughly with the axes of the heart muscle mesh; (c) in general, their greater abundance in hearts of more rapid beat; and (d) their presence also in the striated muscle of the media in the proximal (beating) end of the pulmonary arteries (e.g., mouse).

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A NEW TYPE OF FAT STORING MUSCLE IN THE SALMON, *ONCORHYNCHUS* *TSCHAWYTSCHA*¹

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TWO FIGURES (ONE PLATE)

The king salmon, *Oncorhynchus tschawytscha*, like a number of other fishes, possesses a thin and superficial muscle lying along the side of the body just under the lateral line. This muscle extends the full length of the body of the fish from the pectoral girdle to the caudal peduncle.² It is thickest at the lateral line and becomes gradually thinner as it spreads out in a sheath over both the dorsal and the ventral surfaces of the deep portion of the great lateral muscle. In transverse section the shape of the muscle on each side is that of two scythes placed together base to base. When the muscle tissue is coagulated, as in heating, its dark appearance makes it stand out with prominence. In so far as the king salmon is concerned this dark muscle presents characteristics of peculiar interest which seem to me to justify a special characterization and report.

The dark muscle is a differentiated portion of the lateral muscle mass. Its fibers run in a longitudinal direction. The muscle is broken into short segments, myomeres, by transverse connective tissue bands, the myocommata. In comparison with the remainder of the great lateral muscle, the profundus, the dark muscle is characterized by its relatively small fibers. These fibers are very compactly arranged having a minimum of interstitial connective

¹ Published by permission of the U. S. Commissioner of Fisheries.

² In an anatomical description of the salmon muscles I have given this muscle the name, 'musculus superficialis lateralis.' The deep portion of the great lateral muscle is called the 'musculus profundus lateralis.' Paper now in manuscript.

tissue. The muscle as a whole is marked off from the great lateral muscle by a pretty definite fibrous partition. This separation is not loose enough to be easy of dissection, but when the connective tissue is softened, as in the process of cooking, the muscle very readily separates from the deep portion of the lateral muscle.

I have examined the histological structure of this dark muscle, however, chiefly with special reference to the normal loading of fat. The muscle possesses the usual gross arrangement of sarcolemma, sarcoplasm, and fibrillae. The fibrillae are band shaped and appear in cross section as doubly refractive lines not unlike the appearance of bacilli arranged side by side. These fibrillae vary in size, but are in the neighborhood of from 1 to 1.2μ in cross section in their long diameter. At the surface they are radially arranged with reference to the axis of the fiber, forming a rather definite layer around the circumference of the fiber. But they are irregularly placed throughout the central portion of the fiber, as shown in figs. 1 and 2.

The most striking characteristic of the normal dark muscle is the relatively large amount of sarcoplasm. The sarcoplasm forms thin layers between the doubly refractive lines shown in cross section of the fibrillae, and fills up the spaces where groups of fibrillae are brought in contact, i.e., the angles of Cohnheim's areas. In paraffin sections of adult muscle, both from the young salmon and from mature adults, there is always present in the sarcoplasm a number of clear globules or vacuoles. The number and size of these vacuoles is so great as to obscure the usual relations of the sarcoplasm, fig. 1. The vacuoles are largely in the angles of Cohnheim's areas, but may be present in spaces between the fibrillae in the individual rows. These vacuoles represent spaces from which fat has been extracted during the imbedding process. On the whole the muscle fibers are characterized by resemblance to the more generalized type of striated fiber. Especially in the younger fibers do we find an excessive amount of sarcoplasm both around and between the fibrillae. Other types of striated muscle of the salmon have smaller fibrillae and relatively less sarcoplasm.

There is nothing particularly characteristic of the sarcolemma of the normal dark fiber except that it is strikingly widely separated from the surface of the fiber. Where the sarcolemma is so separated the space between it and the fiber is filled in with definite spherical vacuoles from which fat has been dissolved in the preparation.

There is abundant evidence of the cleavage of these dark muscle fibers in material obtained from the relatively young salmon. This evidence consists in the arrangement of fibers in groups and in the presence of various stages of the separation of fibers, a point chiefly identified by the arrangement and the formation of sarcolemmal partitions and by the disposal of fat in the fibers.

The normal loading of fat

The dark muscle is heavily loaded with fat. The fat is present both between the muscle fibers and within the muscle fibers as shown in fig. 1.

The amount of fat between the fibers is relatively small. It is present in drops from 5 to 20μ in diameter, chiefly in areas which contain blood vessels. The fact peculiar to this muscle and on which I wish to lay emphasis is the presence of enormous quantities of intramuscular fat. I have followed this fat by the special method of staining with scarlet red, Bell's modification of the Herxheimer method, and have confirmed the observations by paraffin sections of both young and adult tissues.

The fat is distributed within the muscle fiber in two regions. First, within the sarcoplasm throughout the substance of the muscle, and second, under the sarcolemma but outside the sarcoplasm. The sarcoplasmic fat is in droplets of extreme variation in size. In the normal mature muscle these fat drops run from a fraction of a micron to as much as 6, or in rarer instances even 10μ in diameter. In one typical young fish the average of the large intramuscular droplets is from 4 to 6μ in diameter. The fat droplets are located at the point corresponding with the angles formed by Cohnheim's areas. Great variety exists as to the size

of the droplets and among these larger drops, sometimes in close approximation to them, will be found numerous smaller droplets. The smallest droplets present are visible only under the oil immersion lens. In numerous instances the smallest droplets are found between the adjacent fibrillae.

In the normal tissue great quantities of fat are also found over the surface of the fiber but under the sarcolemma. That is to say, the fat is stored between the sarcolemma and the muscle substance. There is great variation shown by the individual fibers. Some of them present almost continuous rings of fat droplets, especially in the younger fish, while others show fat around only a small portion of the surface. In the scarlet red stained material it is not always easy to demonstrate this relationship of the fat droplets, but in paraffin material stained by Mallory's aniline blue stain it is easy to confirm the fact that the fat is within the sarcolemma.

The appearance of the fat and its relations within the dark muscle fibers, especially within the sarcoplasm of the fibers, is best shown by the two figures, one of which (fig. 1) gives the positive picture obtained by scarlet red staining of frozen sections of formalin-fixed tissue; the other (fig. 2) the negative obtained by the imbedding method. The presence of fat within muscle fibers is well enough known, but I have thus far found no reference in the literature to any such concentrated loading as is represented in this dark muscle from the king salmon. My general report on this work, together with comparisons with other muscular tissues in the king salmon will give evidence for making the assumption expressed in the title, namely, that we are dealing here with a new and special type of fat-storing muscle.

The functional significance of this type of muscle seems to me to be found largely in the fact of its ability to store and to liberate again such unusual quantities of fat. The muscle has the function of lipogenesis strongly developed. It would seem that it is illustrative of one specific tissue in which Loevenhart's suggestion of a lipogenesis is a specific physiological function. To what extent this function supersedes or displaces the general contractile power, if it does either, remains to be determined.

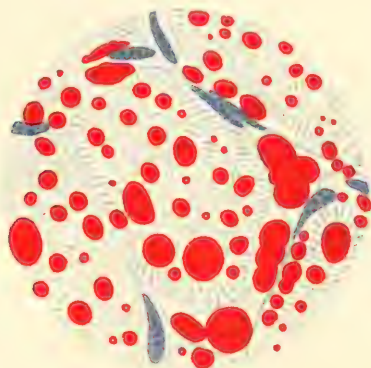
PLATE

PLATE 1

EXPLANATION OF FIGURES

1 A cross section of the superficial lateral or dark muscle of salmon no. 97, a fish from the McCloud River at Baird, California. This preparation was made as a frozen section of material after a fixation of four months in 10 per cent formalin. The section was stained in an alkaline-alcoholic solution of scarlet red fat stain and counterstained with Delafield's haematoxylin. It was mounted in glycerine. The figure shows a striking amount of fat present throughout the fibers with a few large globules between the fibers. Magnification, Leitz ocular 3, objective 7, camera lucida outlines.

2 A cross section of the superficial lateral or dark muscle of fish no. 97, from the McCloud River at Baird, California. The material is stained with Mallory's aniline blue connective tissue stain. The figure is characterized by the large number of clear spaces which represent vacuoles produced by the extracting of the fat in the imbedding process. Magnification, Leitz ocular 3, objective 1/12, camera lucida outlines.



1



2

TYPES OF OSTIA NASOLACRIMALIA IN MAN AND THEIR GENETIC SIGNIFICANCE

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FIFTEEN FIGURES

During a recent investigation on the genesis and development of the nasolacrimal passages in man, my attention was frequently directed to the marked variations that exist, in the adult, in the manner of communication between the ductus nasolacrimalis and the meatus nasi inferior. I, therefore, wish in this note to refer to the principal types of ostia nasolacrimalia found, and to call attention to their probable genetic significance.

LOCATION

At the outset I may say, according to the material studied, that the ostium of the ductus nasolacrimalis is invariably located somewhere on the ventral portion of the lateral wall of the meatus nasi inferior. The variations encountered are due to differences in type, position within the above limits, and to duplication. Notwithstanding that the large series of specimens examined for the substance of this communication invariably presented the ostium of the ductus nasolacrimalis on the lateral wall of the meatus nasi inferior, Geddes¹ reports an unusual and apparently unique abnormality in an Irish male subject of the age of twenty-eight years, in which the ductus nasolacrimalis communicated with the meatus nasi medius. I will refer to this unusual abnormality in a subsequent paragraph.

Within the limits of the ventral portion of the lateral wall of the meatus nasi inferior there is considerable variation as to the

¹ An abnormal nasal duct. *Anatom. Anz.*, Bd. 37, no. 1, 1910.

position of the ostium nasolacrimale. It is located from 15 to 20 mm. dorsal to the limen nasi, and from 30 to 40 mm. dorsal to the naris (anterior naris). Considerable variation also exists in the cephalo-caudal plane: It is frequently found in the most cephalic portion of the meatus nasi inferior, immediately caudal to the attachment of the concha nasalis inferior to the lateral nasal wall. Again we see specimens in which the ostium is 10 mm. (rarely more) caudal to the above point. Between these two extremes we, of course, encounter ostia at various distances caudal to the attached border of the concha nasalis inferior (figs. 4, 6, 9 and 11).

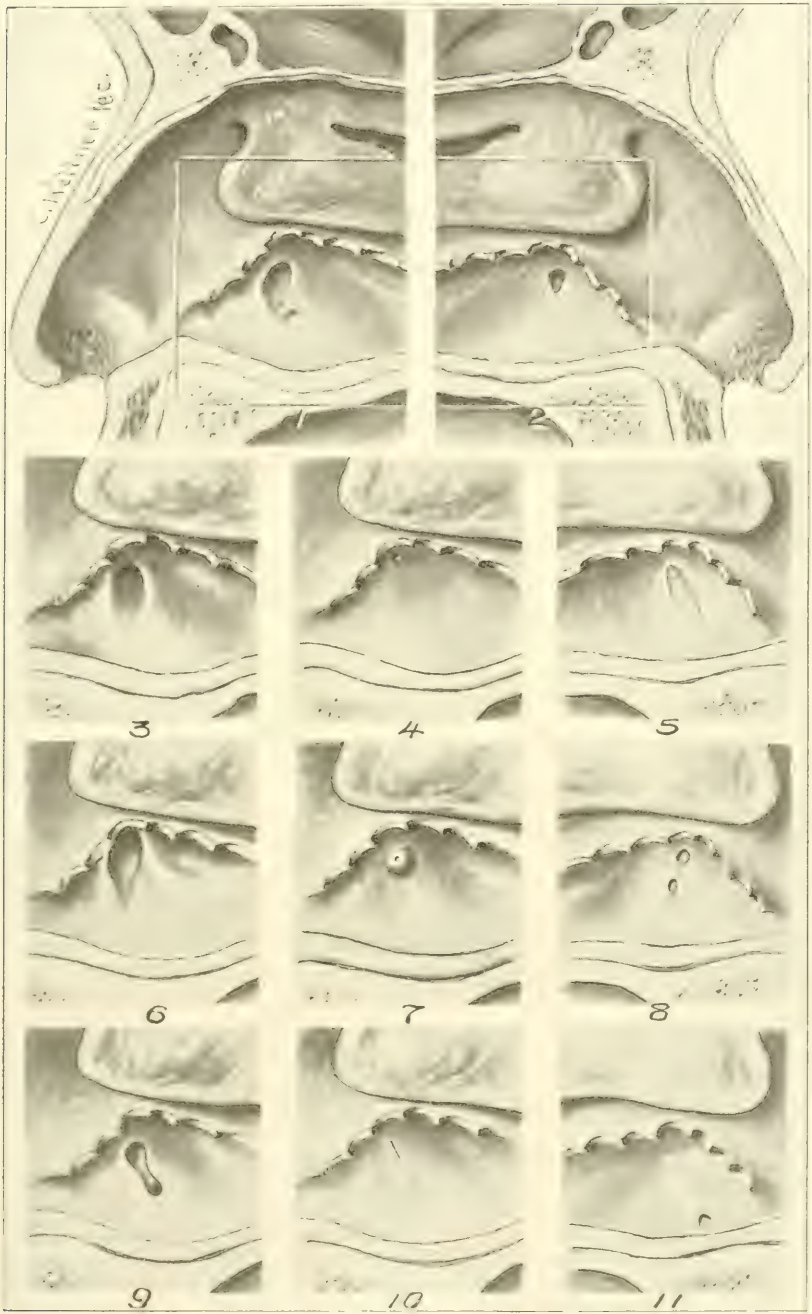
DUPLICATION OF THE OSTIUM

The ostium nasolacrimale is usually a single opening. We, however, encounter specimens in which the opening is duplicated. A triplicity of the ostium was encountered in the series studied. Fig. 8 represents a specimen in which the ostium was duplicated.

TYPES OF OSTIA

The unqualified statements found in some of our text-books, that the ductus nasolacimalis at the point of communication with the meatus nasi inferior, is provided with a valve (plica lacrimalis or the so-called valve of Hasner) is certainly at variance, in many instances, with the real anatomic condition. A study of this ostium or communication in a large series of cadavers at once demonstrates that there is *no unvarying typical form* but that, on the other hand, we are dealing with *several normal anatomic types* of ostia nasolacrimalia. In order to make more comprehensible the several types of ostia encountered in this study it may be well to refer to the illustrations, figs. 1 to 11, in the accompanying plate. The drawings were made from actual dissections

Figs. 1 to 11 Drawings of actual dissections illustrating the various types of ostia nasolacrimalia encountered in this study. The reader is referred to the text for a further consideration of them. The concha nasalis inferior is represented as partly cut away so as to expose for study the manner of communication between the ductus nasolacimalis and the meatus nasi inferior. $\times \frac{1}{2}$.



of the region. The concha nasalis inferior is represented as partly dissected away so as to expose for study the ostium of the ductus nasolacrimalis.

In fig. 5 is represented a fairly common type of ostium. The ductus nasolacrimalis passes through the nasal mucous membrane rather obliquely. The ostium of the duct is rather indefinite and slit-like, and is essentially a potential space. It is guarded by a fold of mucous membrane (*plica lacrimalis*). The ostium continues caudally towards the floor of the nose in a very shallow, gutter-like depression in the nasal mucous membrane—the depression or gutter becoming shallower and shallower until its ultimate disappearance.

Another very common type of ostium nasolacrimale is represented in figs. 3 and 6. The ostium is located immediately caudal to the attached border of the concha nasalis inferior, i.e., at the most cephalic portion of the meatus nasi inferior. It passes more or less directly through the mucous membrane of the nose and in this respect contrasts strongly with the types represented in figs. 5 and 10. The ostium of this type (figs. 3 and 6) is unguarded by folds of mucous membrane, and always presents as a wide, unguarded, open-mouthed and more or less circular opening. This important type of ostium nasolacrimale, of very common occurrence, is not even mentioned in many of our texts. It is easily located and probed, and in all respects stands in marked contrast with the slit-like types. This type of ostium is not provided with a 'valve.'

In fig. 9 is shown a frequent type of ostium. The ostium proper is usually more or less open and somewhat guarded by a *plica lacrimalis*. Extending from the ostium caudally is a rather deep, gutter-like groove which tends to become deeper as it approaches the floor of the nose. The gutter does not disappear by becoming shallower and shallower as in type fig. 5, but it ultimately terminates in a blindly ending pouch in the nasal mucous membrane.

In fig. 10 is represented the extremely narrow, slit-like type of ostium. It is essentially a potential opening and is well guarded by a *plica lacrimalis*. It passes very obliquely through the nasal mucous membrane. This type of ostium is usually located with

difficulty. The narrow slit extends in a cephalo-caudal direction and it contrasts somewhat with the wide slit-like type represented in fig. 5.

Rarely we find an anomalous type of ostium represented in fig. 7. The ostium is located on a raised or nipple-like projection of the nasal mucous membrane.

The ostium represented in fig. 4 is also unusual. It is an extremely small, circular opening located immediately caudal to the attached border of the concha nasalis inferior. The common type of ostium for this position is the wide, unguarded, open-mouthed type.

The other figures represent variations of the principal types already referred to. The normal anatomic types, since they occur so frequently in the series of specimens studied, are represented in figs. 2, 5, 6, 9 and 10. Figs. 4 and 7 are illustrations of anomalous ostia, occurring very infrequently in the series. Figs. 1, 3, 8 and 11 are variations of the normal anatomic types.

GENETIC SIGNIFICANCE OF THE SEVERAL TYPES OF OSTIA NASOLACRIMALIA

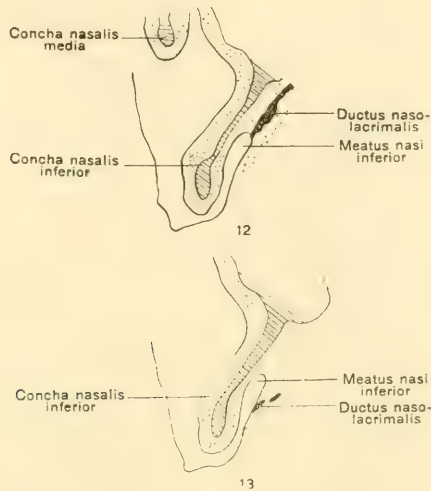
It is well known from the work of many observers that the nasolacrimal passages have their anlage in a thickening of the rete mucosum of the epidermis, along the floor of the rudimentary naso-optic furrow. This solid strand of epidermal cells ultimately becomes entirely isolated from the surface (in man) and for some time is wholly encompassed by mesenchymal cells.² From the ocular end of the isolated epidermal strand of cells develop two sprouts which become the ductus lacrimales (superior and inferior). There is also a sprouting from the nasal end of the strand of cells which in time grows sufficiently to establish coalescence with the mucous membrane of the lateral wall of the meatus nasi inferior. The anlages of the nasolacrimal passages later become canalized.³

The manner of coalescence of the strand of epidermal cells with the nasal mucous membrane especially concerns us with

² J. Parsons Schaeffer, The genesis and development of the nasolacrimal passages in man. *Amer. Jour. Anat.*, vol. 13, no. 1, 1912.

³ *Loc. cit.*

reference to the adult types of ostia nasolacrimalia. The embryology of the nasolacrimal passages demonstrates that the point of coalescence of the strand of epidermal cells with the mucous membrane of the lateral wall of the meatus nasi inferior is inconstant. The place of coalescence may be at the most cephalic point of the meatus nasi inferior (fig. 12). On the other hand,



Figs. 12 and 13 Outline drawings of frontal sections (from human embryos) through the region of the developing nasolacrimal passages. Note in fig. 12 that the strand of epidermal cells, the anlage of the nasal end of the ductus nasolacrimalis, has coalesced with the mucous membrane of the nose at the most cephalic portion of the meatus nasi inferior. In fig. 13 the point of coalescence is farther caudal on the lateral wall of the meatus nasi inferior. The ductus nasolacrimalis is represented in the drawing as solid (see previous paper as to the time and manner of canalization of the duct).⁴

the point of coalescence may be much farther caudal on the lateral wall of the meatus nasi inferior (fig. 13). There is also considerable variation in the ventro-dorsal plane.

The point of coalescence of the strand of epidermal cells with the nasal mucous membrane, of course, determines the position of the adult ostium nasolacrimalale (figs. 6 and 11). The manner of this early coalescence also materially influences the type of

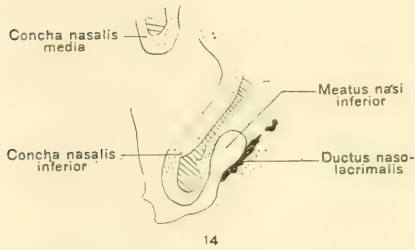
⁴ Loc. cit.

adult ostium: If at the most cephalic point of the meatus nasi inferior, the 'penetration' of the nasal mucous membrane is more or less direct, and the large, unguarded, open-mouthed ostium of the adult would very likely result (compare figs. 6 and 12). On the other hand, if the strand of cells strikes the lateral wall of the meatus nasi inferior obliquely, the resulting ostium is likely to be slit-like and more or less guarded by a plica lacrimalis (compare figs. 5 and 10 with fig. 13).

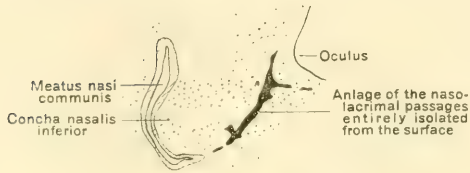
The area of coalescence between the strand of epidermal cells—the anlage of the nasolacrimal passages—and the nasal mucous membrane is, at times, quite extensive (fig. 14). In the process of lumen formation, in such cases, it is reasonable to believe that several ostia, communicating between the ductus nasolacrimalis and the meatus nasi inferior, may be formed. I believe that these extensive areas of coalescence account for most cases of duplication of the ostium nasolacrimale. Intervening bridges of mucous membrane would, of course, remain as the division planes between the several ostia (figs. 8 and 14 should be compared).

In the development of the nasolacrimal passages we frequently see bud-like projections extending from the main strand of epidermal cells (fig. 15). These buds are frequently seen near the meatus nasi inferior. It is reasonable to believe, in some instances, that several of these nasal sprouts establish communication with the meatus nasi inferior. Such a development would account for a duplication of the ostium of the ductus nasolacrimalis. It would also account for the rarer condition in which the several ostia open into independent, short canals which in turn open into the ductus nasolacrimalis. Usually however the bud-like projections from the main strand of cells do not develop sufficiently to establish coalescence with the nasal mucous membrane, but end blindly. The latter would account for the very common diverticula from the adult ductus nasolacrimalis.⁵ Doubtless many of these buds disappear entirely. It is well known that we may also have a duplication of one or both of the ductus lacrimales. This duplication must be explained along similar lines.

⁵ J. Parsons Schaeffer, Variations in the anatomy of the nasolacrimal passages. *Annals of Surgery*, August, 1911.



14



15

Fig. 14 Outline drawing of a frontal section through the developing ductus nasolacrimalis (human embryo). The duct is represented as solid. Note the very extensive coalescence of the duct with the nasal mucous membrane. Compare with figs. 12 and 13.

Fig. 15 Frontal section through the nasal fossa and the anlage of the nasolacrimal passages, from a human embryo aged forty-three days. Note that the anlage is entirely isolated from the surface. The ductus lacrimales have started to sprout from the ocular end of the strand of epidermal cells. The nasal end of the strand of cells has not developed sufficiently to come in contact with the nasal mucous membrane. Especially note the lateral buds from the main strand of cells. $\times 16.5$.

The case of Geddes, in which the ductus nasolacrimalis communicated with the meatus nasi medius, can be explained by a lateral bud from the main strand (fig. 15). In such a case the accessory or lateral bud, instead of ending blindly or developing sufficiently to establish communication with the meatus nasi inferior, established connections with the meatus nasi medius. For some reason or other, the nasal end of the main strand of cells did not establish connections with the meatus nasi inferior. If, on the other hand, the connection was established, the cord not becoming canalized, would in all likelihood undergo resorption caudal to the sprout which established the definitive connections with the meatus nasi medius. That Geddes was dealing with a true portion of the ductus nasolacrimalis and not with a false

passageway is proven by the fact that the lumen of the duct leading from the meatus nasi medius was lined with similar epithelium to that lining the remainder of the ductus nasolacrimalis.

If in the cases, where we have an extensive coalescence between the strand of epidermal cells and the nasal mucous membrane (fig. 14), the whole area of coalescence should become patent, we would readily duplicate some of the other adult types of ostia represented in the plate, figs. 1 to 11, making due allowance for changes in the further development of the ostium.

I wish to take this opportunity for expressing grateful acknowledgment to Professor Kerr for sending me a series of specimens for study from the Cornell anatomical collection. The other material studied was from the Yale anatomical series. Fully two hundred ostia nasolacrimalia were included in this investigation.

THE DEVELOPMENT OF THE AXIAL VEINS AND LYMPHATICS IN TRAGULUS MEMINNA, ERXLEBEN

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FOURTEEN FIGURES¹

MATERIAL

The material on which this investigation is based consists of five embryos of the ruminant ungulate, *Tragulus meminna* (Indian Chevrotain) measuring 5mm., 6 mm., 13 mm., 20 mm. and 23 mm. respectively. These were obtained by exchange from the Smithsonian Institute through Dr. A. Hrdlicka. The series represents the entire embryological material of this form collected during a number of years in Borneo and adjacent islands of the Malay Archipelago by Dr. Abbott of the Institution.

In view of the very great importance of *Tragulus* to the phylogenetic interpretation of the marsupial and placental vascular system, and of the probability that further embryos of this animal will not soon again become available for study, it seems advisable to publish the main results of the investigation based on the material now in hand. This becomes all the more imperative since we possess the complete account of the development of the postcava in embryos of *Didelphis marsupialis* furnished by McClure ('03), and hence have the opportunity of directly comparing the available series of *Tragulus* with the corresponding marsupial stages.

The two smaller (5 mm. and 6 mm) and the two larger (20 mm. and 23 mm.) embryos of *Tragulus* here described proved to be admirably fixed and preserved and well adapted for minute and detailed study. The intermediate embryo of 13 mm. is in a less satisfactory condition and has hence only been used

¹Expense of illustrations borne by author.

to bridge the gaps between the earlier and the later stages in certain regions, in which its preservation permitted definite conclusions.

In a single adult specimen of *Tragul*us, McClure ('06) found that the post-renal division of the post-cava was placed directly in front of the aorta and formed by the union of the common iliac veins, this union taking place ventral to the aorta. These relations correspond to one of the three types of post-cava described by him ('03) in *Didelphis marsupialis* and figured in his plate 2 as no. 8. McClure concludes that this condition allies the venous organization of *Tragul*us more closely to that of the marsupials than to any of the known ruminants. In a verbal communication to the writer, Dr. McClure states that his earlier observations have been confirmed by the subsequent examination of three additional adult specimens of the animal.

Beddard ('07) observed the same relations of the aorta to the post-renal segment of the post-cava in three adult specimens of *Tragul*us. One male adult and three male fetuses in the Columbia collection show the post-renal segment of the post-cava directly in front of the aorta. The conditions in the adult specimen are illustrated in fig. 1. In *Tragul*us the axial venous channel from the confluence of the internal and external iliac veins (fig. 1, 36 and 37) to the renal level consists of two parts; *a*, the common iliac veins (fig. 1, 38) (paired portion of the post-cava, this latter term being used to keep the account in accord with McClure's description) and *b*, the unpaired portion of the post-cava, ventral to the aorta (fig. 1, 39). The unpaired portion is 6.5cm. in length and extends from the confluence of the common iliac veins to the renal anastomosis. The common iliacs are 4.9 cm. in length and extend from the confluence of the internal and external iliac veins to the paired portion of the post-cava. Each common iliac vein receives the sex vein of the corresponding side (fig. 1, 40). *Tragul*us differs, in this respect, from the majority of marsupials in which the sex veins, as has already been shown by Schulte ('07) and Schulte and Tilney ('09), empty into the unpaired portion of the post-cava or into the post-cava and left renal vein.

With reference to the development of the systemic lymphatics the material does not furnish conclusive evidence in all particulars. The organization of the lymph sac, however, is identical with that observed in the domestic cat by Huntington and McClure ('10). The younger embryos present all the characteristics of the primary venous stage, while the older specimens show the several details of structure typical of the late veno-lymphatic, pre-lymphatic and definite lymphatic stages. The systemic lymphatics, at first, are entirely independent of the lymph sacs and only secondarily acquire connections with them in completing the thoracic duct formation. In the specimens examined they showed no genetic relation to the veins, although their exact histogenesis could not be determined on account of the limited material.

The Born method of reconstruction was employed with a magnification of 100 diameters for purposes of topographical study and 200 diameters for obtaining regional detail. All measurements in the embryo were made from sections after fixation upon the slide and, in most cases, computed from figures obtained at a given magnification. None of the embryos was injected.

Venous organization of the 5 mm. embryo (fig. 2)

This embryo presents an imperfectly closed neural tube in the entire spinal region. Its venous channels have a bilateral, symmetrical arrangement, there being four sets of paired trunks, all parallel with the long axis of the body. In addition to these drainage lines there are two plexuses, one, the perimesencephroic plexus surrounding the mesonephros, the other, the umbilico-cardinal plexus which is still active in draining the territory of the body wall into the umbilical and post-cardinal veins.

1. *The umbilical veins (fig. 2, 6).* These are the largest channels present; they are as yet equal in size and show no tendency to lose their bilateral, symmetrical arrangement. No communication has been established with the hepatic sinusoids, so that the two umbilical veins are still independent vessels. They are, to a considerable extent, concerned in drainage of the body

as well as in placental circulation. This is shown by the rich umbilico-cardinal plexus which permeates the lateral somatic wall. Each vein opens into the sinus venosus of its own side in common with the vitelline entrance and forward of the Cuvierian approach (fig. 2, 10).

2. *The omphalomesenteric veins* (fig. 2, 9). These veins, also paired and symmetrical, are smaller than the umbilicals. Although their point of entrance into the sinus venosus is the most mesial and ventral of all vessels entering the heart, the plane of the main trunk passes about midway between the umbilical and post-cardinal veins, so that the course of the vessel as it approaches the sinus is deflected sharply forward and inward. Two divisions of this vessel may be recognized, an intra-hepatic portion which has entered into the formation of the sinusoids and is situated at the dorso-mesial angle of the liver. It thus comes into close proximity to the coelomic angle. The second or omphalic portion passes out upon the yolk sac, leaving the body through the external umbilicus. In this way, each omphalomesenteric vessel, in its intrahepatic portion, has its long axis parallel both with the umbilical and post-cardinal veins, but interposed between it and each of these channels are the mesial branches of the perimesonephroic plexus. No evidence of any distinctly mesenteric branch was found.

3. *The post-cardinal veins* (fig. 2, 5). These are present as a pair of vessels of medium size which have become definite channels throughout their entire length. They hold their typical position dorso-lateral to the mesonephros and still retain part of the plexiform connection with the umbilical veins (fig. 2, 7). This connection becomes greatly reduced as the promontory is reached. The size of the vessel increases as it ascends to become confluent with the pre-cardinal, but before doing so it turns forward almost at right angles to its axial course and by this horizontal arm enters the duct of Cuvier. Immediately below this sharp forward bend, the post-cardinal receives a large tributary from the cephalic extremity of the perimesonephroic plexus. In the greater part of its course many branches from the plexus join this vessel. At some points the vein has

a tendency to reduplicate its channel; the redundant element then lies mesial to it. In the region of the promontory several small dorsal tributaries enter the vessel.

4. *The pre-cardinal veins* (fig. 2, 2). These vessels constitute the fourth pair of symmetrical channels and appear in the typical position of the early stages. The main trunk is parallel to the neural tube and descends toward the Cuvierian duct, into which it enters, after curving sharply forward and inward. It follows the direction of the neural axis cephalad, adapts itself to the cervical flexure, and turns forward nearly at right angles to its descending portion. In this way the vessel may be regarded as presenting two limbs, *a*, an arched or horizontal limb which is assuming the position of the vena capitis lateralis, and *b*, a straight or descending limb in the jugular line. The straight limb receives several small, dorsal tributaries whose development plays an important rôle in the genesis of the veno-lymphatics.

5. *The perimesonephroic plexus* (fig. 2, 8). This forms a dense network around the mesonephros throughout its entire length. It has its greatest density along the ventral surface of the organ. At many points the large mesonephroic sinuses communicate with the plexiform channels. The plexus becomes much reduced in size on the mesial and lateral surfaces of the mesonephros, where an irregular series of anastomosing channels establish communication with the post-cardinal vein. During the course of development, the ventral portion of the perimesonephroic plexus takes part in the evolution of certain axial vessels; the mesial anastomosing branches give rise to the lateral portion of periaortic plexus (fig. 3, 46 and fig. 4, 46).

6. *The umbilco-post cardinal plexus* (fig. 2, 7). This connects the umbilical and post-cardinal veins. It represents the intermediate phase during which the umbilical vein has preserved its capacity as a drainage line for the body wall and is about to give this function over to the post-cardinal. In many places the plexus has already broken down and appears to be selecting the post-cardinal as its ultimate channel.

Such evidence of lymphatic organization as this embryo affords confines itself to the primitive venous fundaments of the jugular lymph sacs. If these elements did not so nearly duplicate the conditions of the early ground plan of the sac in the cat, it would be difficult, without further material, to recognize their true significance. They appear as several dorsal tributaries in connection with the straight portion of the pre-cardinal vein, with the promontory and with the cephalic extremity of the post-cardinal vein. Three of these branches are related to the straight limb of the pre-cardinal, one to the promontory and one to the post-cardinal. The tributaries in relation with the pre-cardinal vein are irregular and have the appearance of redundancies in the vein line. One in particular, the third and most caudal of the series, is larger and more irregular than the rest. Another characteristic of early lymph sac formation is the marked increase in size of the pre-cardinal vein and promontory as they draw together toward the Cuvierian duct. This augmentation does not appear to be in response to the entrance of new tributary lines, but, as in the cat, seems to be a redundant growth of the venous network prior to its differentiation into distinct venolymphatic channels. Upon these grounds, and especially because of their striking correspondence to the conditions observed in the cat, these structures may fairly be taken to represent the elements which determine the early or primary venous stage of lymph sac organization.

The venous organization of the 6 mm. embryo (fig. 5 and fig. 6)

The advance observed in this embryo is characterized by the emergence of a definite sub-cardinal drainage line out of the perimesonephroic plexus and the disturbance of the bilateral symmetry in the umbilical veins. There are five sets of paired venous channels, the general disposition of whose course is parallel to the long axis of the body. The perimesonephroic and umbilico-cardinal plexuses have lost their definite outlines.

1. *The omphalomesenteric veins (fig. 6, 9).* Notwithstanding the fact that the cephalic portion of each vitelline vein has become

involved in the formation of the hepatic sinusoids, it is still possible to recognize two well defined venous channels passing through the liver, parallel with the body axis and situated in the angle formed by the junction of the dorsal and mesial hepatic surfaces. The anterior walls of these channels are subject to great irregularities occasioned by their relations to the hepatic sinusoids; their dorsal and mesial walls are well defined. Each channel is situated opposite the coelomic angle, dorso-lateral to the gut tube, directly ventral to the sub-cardinal vein and ventro-mesial to the post-cardinal vein. The two intra-hepatic vitelline vessels not only drain the sinusoids of their respective sides but communicate with each other by several transverse anastomoses which follow a semi-circular course in front of the gut tube. The most cephalic of these anastomotic channels is the largest and lies at a level a little below the vitelline entrance into the sinus venosus. As it approaches this sinus, each vitelline vein undergoes a change in course, curving outward and forward, then upward and inward to a point slightly mesial to the entrance of the umbilical vein. The intra-hepatic vitelline channels may be traced caudad to a large sinus-like blood space situated immediately below the liver. This large sinus has been greatly augmented by the confluence of the two umbilical veins and the omphalic portions of the vitellines. From its position it may be conveniently referred to as the sub-hepatic sinus (fig. 6, 11), which, in this embryo, receives blood from the large umbilical veins and a single omphalic vessel of medium size. It delivers blood to the hepatic sinusoids, and the two intra-hepatic vitelline channels, having a more ample communication with the channel of the right side. Two veins of small size appear in the gut wall in regions below the liver; their relations identify them as the mesenteric veins. The left mesenteric vein is the larger of the two; it establishes connection with the left intra-hepatic vitelline channel at a distance of 100μ cephalad of that vessel's departure from the sub-hepatic sinus. The right and smaller mesenteric vein joins the left vein by a semicircular anastomosis behind the gut at a point 300μ below the entrance of the latter vein into the left intra-hepatic vitelline channel. The follow-

ing components may be distinguished in the omphalomesenteric drainage line.

a. The omphalic or vitelline element, a single vein which drains the blood from the yolk sac into the sub-hepatic sinus.

b. The intra-hepatic vitelline element, appearing as a right and left channel, of which the right is to persist as the hepatic portion of the post-cava, receiving the hepatic revehent veins and, during intra-uterine life, the ductus venosus; the left channel takes part in the development of the hepatic revehent veins and sinusoids. By its early association with the mesenteric veins, this vessel aids in the formation of the hepatic portal system of post-natal life.

c. The mesenteric elements, appearing in the gut wall as right and left veins, the left communicating with the left intra-hepatic channel, while the right vessel gives its drainage to the left mesenteric by a semicircular anastomosis behind the gut. These latter elements give rise to the definitive portal system.

2. *The umbilical veins (fig. 5, 6)*, These vessels, in greater part, still retain their symmetrical arrangement, but the general venous symmetry of the earlier stage is now to some degree disturbed in the region in which the omphalic portion of the vitelline veins become confluent with the umbilical veins to form the sub-hepatic sinus. The significance of the umbilical veins below the sinus appears to be so different from that above it as to justify the distinction of infra-sinal and supra-sinal portions in either vein. The capacity of the infra-sinal portion is several times that of the supra-sinal. The left infra-sinal segment has increased greatly in size and, in places, is a double channel. The right infra-sinal segment is also double, but for a shorter distance. The supra-sinal segment of the umbilical vein is a relatively slender vessel, extending from the outer side of the sub-hepatic sinus to the sinus venosus. In its entire course cephalad it receives numerous anastomotic branches from the umbilico-cardinal plexus and is thus still largely concerned in drainage of the body wall. It aids in the return of blood from the sub-hepatic sinus and so affords two direct passages

from the placenta to the heart, pending the establishment of the well defined ductus venosus.

3. *The post-cardinal veins (fig. 5, 5).* These vessels occupy the typical post-cardinal position. They may be traced caudad as far as the cloaca and there lose their identity in an indefinite plexus. Followed cephalad, each vessel is seen to attain its maximum diameter at the level of the middle of the mesonephros. They still give evidence of the early influence of the perimesonephroic plexus; in several regions a channel is observed lying parallel to the main vessel and continuous with it above and below. The redundant element may be ental or ectal in position. The genesis of this intermediate cardinal element is not yet entirely clear. Like the sub-cardinal vein, it seems to represent the general process by which definite channels are evolved from more primitive plexus formations along the lines of axial growth and drainage. The cephalic portion of the post-cardinal vein is still intimately related to the supra-sinal segment of the umbilical vein by branches of the umbilico-cardinal plexus. In the region of the promontory, each vessel increases in size and receives a number of dorsal tributaries.

4. *The sub-cardinal veins and the perimesonephroic plexus (fig. 5, 12).* One of the characteristic changes in this stage is the development of a definite sub-cardinal vein out of the perimesonephroic plexus. This vein is a slender vessel with here and there a remnant of the former plexus draining into it. This is particularly the case about the lower pole of the mesonephros where the plexus is still rich and shows a tendency to give rise to other axial lines besides the sub-cardinal channel. Here it is possible to recognize a longitudinal drainage line which is being selected along the inner aspect of the plexus, mesial to the sub-cardinal itself and ventro-lateral to the aorta. This element is in the position of the cardinal collateral channel of McClure. Because of its course and relations, three portions of the sub-cardinal vein may be recognized. 1. The caudal vertical portions 2, the middle or horizontal portion and 3, the cephalic vertical portion. The caudal vertical portion begins as a slender vessel from the caudal extremity of the peri-mesonephroic plexus;

it passes cephalad along the ventro-mesial surface of the mesonephros, having its long axis parallel with the aorta. Upon reaching the level of the sub-hepatic sinus the vessel swings dorsad and slightly lateral so that its axis is now turned almost at right angles to its caudal portion. The position which it now occupies distinguishes it as the middle or horizontal portion of the vein. In this portion of its course the vessel closely follows the direction of the intra-hepatic segment of the vitelline vein as the latter is sweeping dorso-cephalad away from the sub-hepatic sinus. The two vessels are still completely separated by the coelom with a measured distance of 10μ between them. The angle determined by the junction of the caudal and middle portions of the vein lies in a direct line with the intra-hepatic portion of the vitelline vein, so that the projection caudad of the axis of the latter vessel would coincide with the axis of the caudal portion of the sub-cardinal vein, and would thus foreshadow the axis of the future post-cava (on the right side). It is not possible to state that the region of closest approach is the region of the final confluence of these two vessels, but it seems probable from the relations of the sub-hepatic sinus, that the ductus venosus, intrahepatic vitelline and sub-cardinal veins of the right side unite at this, rather than at some more cephalic point. The cephalic vertical portion continues the vein cephalad and is the smallest as well as the shortest of the three divisions. It taps the post-cardinal vein 200μ above the cephalic pole of the mesonephros. It is parallel to the intra-hepatic portion of the omphalomesenteric vein, from which it is separated by a mean distance of 20μ .

5. *The pre-cardinal veins (fig. 5, 2) and veno-lymphatics.* The general arrangement of the pre-cardinal veins has undergone no marked change. The vessels still present an arched and a straight limb. There has been, however, considerable modification in the character of the dorsal tributaries. These have expanded and become confluent in several places. The expansion has continued after confluence has taken place and the channels have been converted into irregular spaces. The four dorsal tributaries to the pre-cardinal vein have all united, while

the tributaries coming into the promontory and cephalic portion of the post-cardinal have joined to form a large blood space. The tendency of the pre-cardinal and post-cardinal veins to increase in size as they approach the duct of Cuvier is still evident. On the right side there are clear signs of a beginning fenestration in the base of the pre-cardinal, thus indicating the inception of the para-pre-cardinal line.

Marked changes are met with in passing from the primitive organization of the 6 mm. embryo to the condition of the 20 mm. embryo. As touching upon the stages intermediate between the two, reference will be made to the 13 mm. specimen. A noteworthy acquisition in the 20 mm. embryo is found in the now fully developed lymph sacs, and also in the formation of the main segments of the systemic lymph channel. These two elements are as yet entirely distinct and separate. The plan of venous drainage foreshadowed in the 6 mm. embryo has been carried well on towards completion. In this process a single post-cava has been acquired as far as the inter-renal anastomosis while below that level, two large symmetrical channels, representing the cardinal collateral veins, coöperate in the formation of a double post-cava.

The lymphatic organization in the 20 mm. embryo (fig. 7)

In the 20 mm. embryo, the jugular lymph sacs (fig. 7, 25) are situated in the neck region, one on either side, on the lateral aspect of the great vessels and nerves. Each sac has a general wedge shape, with its base looking inward and forward, while its edge lies between the vertebral column and the dorso-lateral surface of the body. Its greatest diameters are attained about midway between its cephalic and caudal poles. The 3rd, 4th, 5th and 6th cervical ganglia lie dorsal to the sac. The third cervical nerve traverses its cephalic pole, the sixth nerve passes beneath its caudal pole, while the fourth and fifth nerves go directly through it. The right sac is 1.61 mm. in length, the left sac 1.73 mm. The maximum ventro-dorsal diameter of the right sac is 1.68 mm., that of the left sac being 1.6 mm. The

maximum transverse diameter of the right sac is .54 mm., that of the left sac is .48 mm. It will be convenient to describe the structure as having a body with caudal and cephalic processes.

The body of the lymph sac (fig. 7, 25). This portion of the sac appears as a prominent feature of cross sections in the neck region. The walls of the sacs are thinner than those of the veins. The mesial and ventral walls are smooth and regular, the lateral wall presents many irregular projections. The broad ventral portion of the sac lies directly back of the jugular vein (fig. 7, 35). Mesially it is in relation with the carotid artery, the vagus and sympathetic nerves, but separated from them by an extensive plexus of venous channels draining into the jugular system. In its subsequent development, this plexus allies itself with the lymphatic system.

Processes of the sac. The contour of the sac becomes irregular in several regions by prolongations from its walls. These prolongations serve as the processes by which the sac acquires its ultimate connections with the venous system and the systemic lymphatics. From the cephalic pole a large number of processes reach upward into the head region and end blindly. Some of these prolongations serve to connect with the systemic lymphatic trunks from above. The caudal processes are larger and show a more definite arrangement. Their number and disposition differ on the two sides. This difference depends upon the relations which each sac bears to the adjacent veins. The left sac is still freely connected with the jugular vein; the right sac is entirely cut off all venous connection. The left sac is typical of the conditions in the late veno-lymphatic period. The right sac affords a good example of the pre-lymphatic stage. Due to its free communication with the left jugular vein, the left sac is filled with blood. Caudally, it is divided into two main processes, one of which turns mesad and ventrad in the direction of the jugular vein, the ventro-mesial process; the other continues caudad in line with the general axis of the sac, the dorso-lateral process. The ventro-mesial process enters directly into the vein, On the right side the sac has already been emptied of blood and has lost connection with the jugular vein. The dorso-lateral process (fig. 7, 26) is present on each side. It does not connect

directly with the veins but proceeds caudad to form three other processes, namely, the lateral process or subcutaneous duct, the dorsal descending process or thoracic duct approach (fig. 7, 26a) and the ventral descending or broncho-mediastinal approach. The ventral and dorsal descending processes become divergent immediately above the cephalic vein; the ventral process passes caudad on the ventro-lateral aspect of the jugular vein to join the broncho-mediastinal systemic channels on the right side. On the left this connection is not completed. The dorsal descending process extends a short distance caudad, dorso-lateral to the thyreo-cervical artery to end blindly.

Organization of the systemic lymphatic drainage lines. The evidence concerning the development of the lymph sac in *Tragulus* shows that this structure is derived from the venous system. In its early stages the sac is wholly independent of the systemic lymphatics. Subsequently it joins with the systemic lymphatic channels, and thus serves as the connecting link between these channels and the venous system. In the material studied it was impossible to discern any genetic relation between the developing systemic lymphatics and the veins. Such regions as gave the earliest pictures of the organization of the systemic lymphatics revealed these elements as independent mesenchymal spaces, at first presenting the form of a plexus. By a process of expansion and confluence this plexus comes to form definite channels. In the 20 mm. embryo development has advanced too far to offer anything that is conclusive as to the actual histogenesis of these lymph spaces. The process referred to as confluence and expansion is not carried on with the same degree of rapidity or effectiveness in all regions of the embryo; it confines its activities to certain selected districts which remain, for some time, separate. The systemic lymphatic channels arise from three major segments which, because of their relations to the venous system, may be termed the azygos, pre-azygos and post-azygos segments, corresponding to similar segments in the cat as recently described by Huntington ('10).

1. *The azygos segment* (fig. 7, 28). It is in this segment that the systemic lymphatic channels have attained their greatest development. Longitudinally the segment reaches caudad from

the azygos-Cuvierian junction to a level slightly above the inter-renal anastomosis. It is notable for its unusual size as well as for the striking resemblance it bears to the reptilian type of axial lymph channel (fig. 9, 28). On approaching its cephalic extremity the channel breaks up into a rich plexus (fig. 8, 50). The same is true of its caudal extremity. The vessel on the left side is the larger. It is situated ventro-mesial to the azygos vein and dorso-lateral of the aorta. The right channel holds the same relative position. After proceeding a short distance caudad these two parallel channels rapidly expand and become confluent across the median line behind the aorta. The azygos segment interposes itself between that vessel and the two azygos veins, which latter are connected with each other by anastomosing vessels passing behind the aorta. The relations of the azygos segment to the other large channels are illustrated in cross section in fig. 9.

2. *The post-azygos segment* (fig. 7, 29). This portion of the systemic lymphatic channel pertains to the abdominal region where it appears as an irregular vessel, at times double and again fused, behind the aorta. It continues downward in this condition to the iliac bifurcation where it becomes a considerably dilated, single vessel again bifurcating at its caudal extremity. At the point of this bifurcation it alters its relations in such a manner as to lie lateral to the iliac vein, taking up the ultimate position of the ilio-lumbar lymphatic trunks. At its cephalic extremity it ends in a plexus which has already established several connections with the plexus at the caudal extremity of the azygos segment.

3. *The pre-azygos segment* (fig. 7, 30 and 31). In this portion of the systemic lymphatic channel the process of expansion is apparently less active than elsewhere. Two general lines of development may be traced, one on the right in relation to remnant of the right pre-cardinal vein and aorta, the other on the left in relation to the large brachio-cephalic arterial trunk. Each line presents a cephalic and caudal element. On the right side the caudal element communicates with the plexus at the cephalic end of the azygos segment (fig. 8, 50), and from here extends cephalad

between the aorta and pre-cardinal vein as far cephalad as the arch of the aorta. The cephalic element begins as a plexus at the bifurcation of the brachio-cephalic artery and continues cephalad dorso-lateral of the jugular vein as far as the jugulo-subclavian junction. At this point it is separated by an interval of approximately 0.1 mm. from the blind end of the dorsal descending process of the lymph sac (thoracic duct approach). The pre-azygos segment on the left side is even more divided, for although it presents a cephalic and caudal division, each of which appears as a clear cut channel, it was impossible to detect any connection between these two divisions on the one hand and the azygos segment on the other. It ends blindly above and appears to have no connection with the thoracic duct approach of the sac, from which it is separated by an interval of about 0.1 mm. Its caudal end is also independent of any connection. The caudal division begins as a distinct channel at the derivation of the internal mammary artery and extends almost as far caudad as the junction of the brachio-cephalic trunk with the aortic arch. So that, above the azygos segment, the line along which the thoracic duct is destined to develop its connection with the duct approach of the lymph sac consists of two as yet independent elements, the cephalic and caudal divisions of the left pre-azygos segment.

A summary of the systemic lymphatic organization shows that the following elements must be recognized in the future line of the thoracic duct:

(a). The azygos segment (figs. 7, 28 and 9, 28). A spacious sinus-like channel, in places entirely surrounding the aorta and ending at either extremity in a plexus, in many regions resembling the peri-aortic lymphatic sinus of reptiles.

(b). The post-azygos segment, (fig. 7, 29), a series of alternating plexiform and sinus-like channels, connected cephalad with the caudal plexus of the azygos segment.

(c). The pre-azygos segment, (fig. 7, 30 and 31), consisting on the right side of two independent divisions, the cephalic and caudal, of which the latter is connected with the cephalic plexus of the azygos segment while the former has not yet established

communication with the thoracic duct approach of the lymph sac. On the left side, the two divisions are also present, but neither has made connection with other parts of the lymphatic system.

Lymphatic organization in the 23 mm. embryo

Axial lymphatic organization has been carried to its consummation in the 23 mm. embryo. The imperfectly crystallized conditions of the next younger specimen have already marked out the line along which this development would proceed.

Body of the lymph sac (fig. 10). The body of the sac on both sides lies in front of the 3rd, 4th, and 5th cervical ganglia, the 6th ganglion and the interspace above it lying entirely below the caudal limit of the vesicle. The long body axis now bears the ratio of 17 to 1 to the long axis of the lymph sac, whereas in the 20 mm. embryo this ratio was 10 to 1. Measurements of the sac as computed from the mounted sections show that this decrease in size is not merely relative but absolute, as the following values of the long axes show:

	23-MM. EMBRYO	20-MM. EMBRYO
	<i>mm.</i>	<i>mm.</i>
Right sac.....	1.49	1.67
Left sac.....	1.61	1.73

The ventro-dorsal and transverse diameters also show a decrease. That this attenuation has chiefly affected the more ventral portion of the sac is shown by the fact that the two nerves (4th and 5th cervicals) which in the 20mm. embryo passed through the sac have now freed themselves, while it also appears that the process which released them has at the same time produced a neck in the sac itself. By this neck the sac approaches the jugular vein and attains its ultimate systemal lymphatic and venous connections. It will be obvious, therefore, that this cervical portion corresponds in general to the caudal extremity in the sac of the next younger specimen.

Processes of the sac. Several of the cephalic processes may be traced for a considerable distance into the head region and un-

doubtedly denote the connections of the sac with lymphatic trunk lines of the head. The main interest with reference to these processes centers about those which are derived from the caudal extremity or what may be termed the cervix of the sac. Upon reaching the level marking the entrance of the cephalic vein into the jugular, the cervix of the sac breaks up into four processes, namely:

1. The dorsal process which extends dorsad accompanying the cephalic vein and receives the dorsal somatic tributary of the sac, the subcutaneous duct.

2. The mesial process, the now much reduced primary veno-lymphatic connection which extends mesad dorsal to the jugular vein but does not tap into it.

3. The dorsal descending process which in the 20 mm. embryo was designated the thoracic duct approach and which has now acquired its full connection with the preazygos segment of the duct, especially on the left side. This process descends along the dorso-lateral surface of the thyreo-cervical artery.

4. The ventral descending process which affords communication with the broncho-mediastinal channels. It appears in a position ventral to the thyreo-cervical artery. As this process enters the sac it forms an acute angle with the dorsal descending process which lodges the cephalic vein as it is opening into the jugulo-subclavian junction. In addition to the broncho-mediastinal approach, the ventral descending process has developed still a third, the jugulo-subclavian approach. This is a slender prolongation from the mesial side of the process which, upon reaching the jugulo-subclavian angle, forms a direct communication with the venous system. This is the so-called secondary venous tap of the lymphatic into the venous system and by its establishment determines the transition from the prelymphatic to the lymphatic stage. The secondary tap is made in a characteristic manner. From the wall of the vein at its jugulo-subclavian junction a process which has the appearance of a tubular redundancy pushes its way cephalad between the lateral vein wall and the mesial wall of the sac. After a short distance it meets and opens into the jugulo-subclavian approach of the lymph sac thus producing a channel between the latter and the

venous system. The venous orifice of this duct-like structure is wide; the saccular orifice is elongated and narrow, while the duct itself is placed between the walls of the sac and the vein. This arrangement makes it appear that distension of the vein would act on the jugulo-subclavian approach in such a way as to produce a valvular effect. By this means the sac has acquired its ultimate connection with the venous system (fig. 11, *A*, *B*, and *C*).

The systemic lymphatic drainage line. The transition from the conditions of the systemic lymphatic organization of the 20 mm. embryo to those of the 23 mm. is characterized by a confluence of the several previously established segments, with the result that the thoracic duct is now a continuous channel and at the same time has upon the left side, acquired connection with the lymph sac.

The azygos segment. This portion of the systemic lymphatic line gives evidence of the least change of character. It is still a capacious channel situated dorsal to the aorta, in places surrounding the vessel. If changed at all, it has somewhat lost in capacity, especially because its periaortic plexuses are less rich and numerous. In relation to the azygos veins, it still is interposed between them and the aorta. The plexuses which, in the early conditions, were observed at its cephalic and caudal extremities have given place to definite channels (fig. 12). From the cephalic plexus there has arisen a single large trunk which is paralleled by a second small lymph vessel, both of which pass over into the pre-azygos segment. The caudal plexus develops two channels which communicate with the post-azygos segment.

Pre-azygos segment (fig. 12). Here the greatest change has occurred, for the plexiform and irregular channels of the earlier pre-azygos segment have become defined as two parallel trunks. The larger of these is the more constant and apparently represents the main line of drainage. Upon the left side the confluence of the several divisions of the pre-azygos segment has been carried so far as to form a complete connecting vessel in communication with the dorsal descending process of the lymph sac of that side. On the right side this confluence is not as com-

plete. The cephalic division of the pre-azygos segment has met and fused with the thoracic duct approach of the sac. The caudal division is still independent.

Post-azygos segment (fig. 10, 29). This segment has also attained more definite outline. Its caudal dilatation is larger and its ilio-lumbar appendages more extensive, so that upon the left side there is an uninterrupted thoracic duct line, which has resulted from the fusion of the pre-azygos, azygos and post-azygos segments. The right duct line is still incomplete. The ventral descending process of the sac on both sides has already established connection with the truncus broncho-mediastinalis. In the main, this trunk is still a dense plexus situated ventral to the thymus; in several places, however, it loses its plexiform character to become a distinct channel. Retaining these general relations to the thymus; it passes to the caudal extremity of that organ, where it undergoes considerable reduction, but may be traced across the aortic arch to the root of the lung.

THE VENOUS ORGANIZATION IN THE LATE EMBRYONIC STAGES

The advance in the venous organization in the 20 mm. embryo depends on a modification in the relations between the umbilical and omphalomesenteric veins. Coalition of the right sub-cardinal with the right omphalomesenteric has produced a definite post-caval system as far caudad as the renal anastomosis. Below this level the cava arises in a manner somewhat different from that observed in the majority of mammals already studied.

Renal anastomosis and cardinal collateral veins

Upon reaching the level of the kidneys, the post-caval drainage line becomes greatly expanded to form a large, irregularly quadrilateral channel situated in front of the aorta. This large channel establishes the renal anastomosis and, from its position, may be termed the inter-renal segment of the post-cava. It presents two cephalic and two caudal angles. From its right cephalic angle the post-caval drainage line is continued toward the heart by means of the right sub-cardinal vein. Its left cephalic angle receives the left suprarenal vein. The renal vein

enters the inter-renal segment immediately below the cephalic angle (fig. 13, 57).

Two vessels of equal size enter the inter-renal segment, one at either caudal angle. They are practically parallel to each other, are placed ventro-lateral to the aorta and separated from each other by a mean distance of 60μ . Caudally they arise from the junction of the internal and external iliac veins. The proximity of these two parallel channels to the median line, their position ventral to the aorta and mesial to the ureters makes it probable that they are derivatives of the ventro-mesial element of the peri-mesonephroic plexus and hence should be considered the cardinal collateral channels (fig. 13, 21).

Immediately above the entrance of each cardinal collateral vessel, a vein from the mesonephros empties into the inter-renal segment. This mesonephroic vein subsequently becomes the sex vein and will hereafter be referred to by that term (fig. 13, 40).

Caudad of its sub-cardinal portion, the axial drainage line is therefore made up as follows, beginning from the confluence of the internal and external iliac veins.

1. The two cardinal collateral veins, constituting the so-called paired portion of the post-cava (fig. 13, 21).

2. The inter-renal segment, situated directly ventral to the aorta and between the kidneys. It receives the cardinal collateral and sex veins at its caudal angles while the renal veins empty into it by its cephalic angles. At its right cephalic angle it passes over into the sub-cardinal portion of the post-cava (fig. 13, 59).

The relative dimensions of the two portions change considerably in the further development of the post-cava; the following tabulation gives the length of the two segments in the older embryos as compared with the adult conditions.

	20-MM. EMBRYO	23-MM. EMBRYO	ADULT
	mm.	mm.	mm.
Length of inter-renal segment.....	0.71	0.75	65
Length of paired portion (cardinal collateral).....	0.14	1.25	49
Distance between renal and sex veins.....	6.26	0.30	54

From these figures it appears that the ratio of the inter-renal segment to the paired portion of the cava (cardinal collaterals) is 1 to 1.6 in the 20 mm. embryo as against 1 to 0.75 in the adult animal. In other words, if the increment of growth has remained constant in the inter-renal segment, it has been reduced one half in the cardinal collateral vessels while passing from the 20 mm. stage to adult. These facts make it clear that longitudinal expansion of the inter-renal segment plays an important rôle in determining the position and relations of the post-renal segment of the cava. This assumption is further borne out by the fact that the distance between the sex veins and the renal veins progressively increases in passing from the embryonic stages to the adult.

The pre- and post-cardinal veins

The pre-cardinal veins may still be identified almost in their entirety even in these late stages. In the greater part of the neck they constitute a pair of channels, the internal jugular veins. High up in their cephalic portion, they receive a short trunk which has resulted from the confluence of the temporo-facial and internal maxillary veins. This trunk represents the external jugular vein. As the two internal jugular veins approach the thorax they become confluent and form a single large vessel. It is into this confluent element that the sub-clavian, cephalic, internal mammary and vertebral veins enter. On passing into the superior mediastinum the single vessel again becomes a double channel. The vessel on the right is much the larger. This portion of the jugular system represents the pre-cava which, after becoming much reduced in size, enters the heart. The left channel proceeds further caudad and finally joins the left post-cardinal vein to form the duct of Cuvier.

The left post-cardinal vein participates in the formation of the left azygos vein. During this process that redundant cardinal channel, earlier observed in the perimesonephroic plexus and referred to as the intermediate element, seems to take a prominent part. In the cephalic portion of the azygos major vessel, the original post-cardinal channel determines the ultimate

vein line, but in those regions corresponding to the perimesonephroic plexus of the younger stages, the intermediate element is obviously the selected channel. The relations of this element to the aorta in the 6 mm. specimen indicate the future line of the azygos vessel. In the 13 mm. embryo the intermediate element has become a more prominent channel than the post-cardinal. A right azygos vein, corresponding in position to the left vessel, is formed in like manner, except that it establishes its ultimate drainage connections by a series of cross anastomoses with the left vein. These anastomoses, at first, are diffuse and, in the 20 mm. embryo, appear as irregular channels between the two azygos lines. The most cephalic channel is well defined and larger than the rest. In the 23 mm. embryo the formation of distinct cross vessels has been carried much further so that these inter-azygos connecting lines are now arranged in segmental series, are twelve in number, and occur at the junction of each dorsal segmental vein with the azygos vessel of its respective side (fig. 10, 62). Another set of anastomosing vessels serves to connect the two azygos channels with the sub-cardinal and cardinal collateral lines. These anastomotic vessels are placed along the sides of the aorta and thus correspond in their relations to the mesial branches of the perimesonephroic plexus observed in the younger stages. Two sets of these vessels may be distinguished, a cephalic anastomosis which traverses the anlage of the supra-renal body and undergoes a gradual reduction in passing from the 13 mm. to the 23 mm. stage. This plexus forms a connection between the azygos vein and the corresponding side of the post-cava in its inter-renal segment. The second plexus is much more extensive. It establishes a communication between the azygos veins and cardinal collateral portions of the post-cava (fig. 14, 61). The significance of this connection in its bearing upon the possibilities of post-caval formation will subsequently be discussed. That it gradually diminishes in significance as growth proceeds is witnessed by the fact of its actual reduction in passing from the 13 mm. to the 23 mm. embryo. The post-cardinal veins thus concern themselves with the azygos system, allowing the selection of the caval drainage line to fall

upon the other channels. The venous plexus between the pre-aortic and post-aortic veins becomes so extensive as to completely invest the aorta except for a small interval immediately in front, on either side of the median line. As it approaches the levels marking the derivation of the hypogastric arteries the plexus becomes more voluminous and finally communicates with the iliac trunks.

From the conditions of these embryonic stages it is obvious that the venous return from the internal and external iliac veins depends upon a large peri-aortic plexus rather than upon discrete venous channels. The increasing prominence of certain axial channels in this plexus clearly indicates the process by which the plexus itself is to be replaced by veins in the line of the longitudinal growth of the body. The course of these axial channels is parallel to the long body-axis; two are placed ventro-lateral to the aorta, the cardinal collateral veins; two are situated dorso-lateral to the aorta, the post-cardinals. At the indeterminate stages represented by the 20 mm. and the 23 mm. embryos the internal and external iliac veins may select one of several possibilities for the continuance of their drainage lines toward the heart. They may choose either or both of the post-cardinal veins to the exclusion of the cardinal collaterals, so that the venous blood would reach the inter-renal segment of the cava by the plexiform channels of the inter-renal post-cardinal plexus. They may select both cardinal collaterals to the exclusion of the post-cardinals and so establish a communication with the inter-renal segment of the cava. The adult conditions, showing that the post-renal segment of the cava is pre-aortic in position, clearly demonstrate that the selection has fallen upon the cardinal collateral veins and that the post-cardinals play no part in the formation of this portion of the cava.

These possibilities of selection which must be reckoned with in discussing the post-renal cava in *Tragulus* emphasize again the statement of Schulte ('09) that

homonymous venous channels are not necessarily morphological equivalents but are rather homodynamous, agreeing in function because they drain similar areas:—and it thus appears that the anatomical name of

veins designate not morphological but physiological units. The term post cava only indicates a hydro-dynamic line. The variously named cardinals are merely dilated portions of the reticulum along the major hydro-dynamic lines, which, responding to the large volume of blood they transmit, dominate the picture.

Although the facts cited above account for the acquisition of a pre-aortic post-renal segment of the cava in the adult *Tragulus*, they do not furnish a complete explanation of the process by which the ultimate relations of this vessel are attained. In the 20 mm. and 23 mm. embryos the post-renal segment of the cava presents two portions, each of which is pre-aortic in position, namely, the paired portion and the unpaired portion. The unpaired portion in these stages, constitutes about one-third of the entire post-renal segment of the cava (fig. 13, *A* and *B*). In the adult, while the paired and unpaired elements still enter into the formation of the post-renal segment, their proportions have greatly changed. The unpaired portion instead of being one-third as long as the paired portion is now six times longer. In other words five-sixths of the post-renal segment of the cava is represented in the adult by a single unpaired pre-aortic channel, the remaining one-sixth being represented by the paired portion (fig. 1). This marked change in proportion may be due to one of three possibilities; 1, the fusion of the two cardinal collateral veins across the median line in the cephalic two-thirds of their course; 2, a caudal migration of the angle of confluence of the cardinal collaterals; and 3, the longitudinal expansion of the inter-renal segment alone. The position and relations of the sex veins considerably lessen the difficulties in deciding which of these processes is the active one. It has already been shown, in the older embryos, that the renal veins mark the cephalic limits of the inter-renal segment while its caudal limits are indicated by the sex veins. The portion of the cava between these limits, therefore, must be considered the inter-renal segment in all stages. Upon this basis, the change in proportion of the two elements of the post-renal cava, observed in passing from the embryonic to the adult stages, may be explained by the longitudinal expansion of the inter-renal segment. The ultimate wide

separation between the renal and sex veins seems to indicate that this was the process by which the post-renal cava has been changed from an embryonic channel in greater part paired to an adult vessel in greater part unpaired. The probability of this explanation is further sustained by the fact that the increment of growth from the early stages has favored the inter-renal segment (see page 213). The relations between the length of the post-renal segment of the aorta and that of the inter-renal segment of the cava also show changes which are significant in this connection. The measurements of the aorta were taken from the point of derivation of the right renal vein to the iliac bifurcation; those of the inter-renal segment from the entrance of the right renal vein to the point of confluence of the two cardinal collateral veins.

In the 20 mm. embryo the length of the inter-renal segment of the cava was 0.33 that of the post-renal segment of the aorta. In the 23 mm. embryo this value has increased to 0.40 and in the adult to 0.87. Thus there has been a relative increase in the rate of growth in the inter-renal segment of the cava as compared with the post-renal segment of the aorta.

The change in the relations of the sex veins and arteries is further evidence of this relative increase in the inter-renal segment of the cava. Both of the older embryos show the sex veins and arteries in close relation to each other (fig. 13, *A* and *B*). The veins enter the inter-renal segment practically in common with the entrance of the cardinal collateral veins. The sex arteries arise separately from the aorta at a level only slightly caudal to that of the veins. The adult specimen in the Columbia collection shows the sex arteries arising from a short common trunk given off from the aorta at a point 1.5 cm. above the iliac bifurcation, while the sex veins enter the common iliac veins (paired portion of the post-cava) 4 mm. below the point of entrance of these latter channels into the unpaired portion of the post-cava (fig. 1). Thus the sex arteries, which in the embryo arise from the aorta caudad of the sex veins, in the adult arise from a level distinctly cephalad of these veins. This marked change in relations appears to have its explanation in the relatively more rapid

longitudinal growth of the inter-renal segment of the cava as compared with the post-renal segment of the aorta. The shifting of the sex veins from their more primitive point of inosculation may be due to a caudal migration of the angle of confluence of these vessels with the post-renal segment of the cava or it may be the result of certain mechanical changes due to the caudal migration and descent of the testis.

The intra-hepatic portion of the right omphalo-mesenteric vein has gained ascendancy over all the venous spaces of the liver and appears as a definite channel situated in the right dorso-mesial angle of that organ. It constitutes the hepatic portion of the post-cava. At the cephalic pole of the liver the vessel is large, receiving, in this region, the two major revehent trunks which drain the hepatic sinusoids. Immediately below the inosculation of these revehent vessels the cava diminishes in size, taking up as it proceeds caudad, several lesser, hepatic revehent tributaries. When the caudal pole of the liver is reached the vessel swings slightly mesad and dorsad, to pass over into the sub-cardinal portion of the cava. The mesenteric portion of the omphalomesenteric vessel has now become the portal vein and drains into one of the largest advehent branches of the umbilical channels. The post-caval drainage line thus utilizes the right intra-hepatic portion of the omphalomesenteric vein in passing through the liver, and the right sub-cardinal vein as far caudad as the inter-renal segment.

The umbilical drainage system presents itself as the typical single channel of foetal life. It makes its way through the umbilical fissure of the liver and then enters that organ. In the liver it breaks up into the rich plexus of the umbilical portal system. The ductus venosus is given off from one of the main stems of this plexus and passes obliquely upward to enter the post-cava in common with the confluence of the major hepatic revehent veins. These observations apply equally to the 20 mm. and 23 mm. embryos.

SUMMARY

The development of the axial lymphatics in *Tragulus* presents the following characteristics:

1. Two distinct anlagen, one for the lymph sac and the other for the systemic lymphatics.

- a. The lymph sac is derived from the venous system; passes from the primary venous into the veno-lymphatic stage, at the end of which period it loses all connection with the veins and so enters upon its pre-lymphatic stage. Ultimately it establishes a secondary connection with the venous system and so becomes definitely lymphatic.

- b. The axial systemic lymphatics develop in three distinct portions, namely the azygos, pre-azygos and post-azygos segments. The exact histogenesis of these segments could not be determined, but they convey the impression of plexiform channels arising independently in the mesenchyme and then rapidly expanding into discrete axial vessels. No connection between these segments and the veins was observed at any point. By confluence the segments became integrated to form the axial systemic lymphatics.

2. The final union of the two distinct anlagen determines the completed axial lymphatic line. In this manner the lymph sac becomes intermediary in establishing a communication between the venous and peripheral lymphatic system, a mode of organization which resembles that of the cat and so, no doubt, the general ground plan in mammals.

Advancing from the stage of symmetrical channels, the venous development is much concerned with modifications in the perimesonephric plexus. This vascular network, bearing intimate relation to the mesonephros, is subsequently converted into such distinct channels as the sub-cardinal and cardinal collateral veins which have adapted themselves to the general line of axial growth.

These facts seem to sustain the proposition that all definite venous channels have their inception in a plexus and emerge from this plexus as definite veins, under the influence of certain hydro-dynamic factors, which are in the interest of most efficient

venous return. Lest it be argued that the perimesonephroic plexus from which the above named vessels arise is a special case in *Tragulus*, it may be stated that this plexus has already been described by Brown ('11) in cat embryos, and has been observed by the writer in several sauropsid forms (chick and scleroporus embryos). The organization of the post-renal segment of the cava also appears to be an instance of the emergence from a plexus of selected axial channels; in this case the channels happen to be the cardinal collaterals. The plexus itself surrounds the aorta; the cardinal collateral vessels mark its ventral limits; the dorsal limits are formed by the post cardinals. A pre-aortic post-renal segment of the cava is established in part by the selection of the cardinal collateral veins as axial channels and in part by a marked longitudinal expansion of the inter-renal segment. These conditions observed in *Tragulus* definitely ally its post-cava with the marsupial type. The similarity thus established between the venous organization of this aberrant ungulate and that of the marsupials has a clear phylogenetic significance. It does not, however, shed much light on the more fundamental problems involved in the development of the post-cava. In fact, it merely serves to open the question as to what hydro-dynamic and other mechanical factors must control the selection of the ultimate venous drainage channels in the axial line of the body.

In conclusion, the writer desires to acknowledge his indebtedness and express his appreciation to Professor Huntington for his direction and assistance in preparing this paper.

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PLATE 1

EXPLANATION OF FIGURE

1 Adult *Tragulus meminna* from a dissection in the study collection of the Department of Anatomy, Columbia University, showing the pre-aortic position of the post-cava. 24, aorta; 34, ureter; 36, internal iliac vein; 37, external iliac vein; 38, common iliac vein (paired portion of post-cava); 39, unpaired portion of post-cava; 40, sex vein; 41, spermatic artery; 42, caudal vein; 43, common iliac artery; 44, external iliac artery; 45, internal iliac artery; 57, renal vein; 58, renal artery; 61, caudal artery.

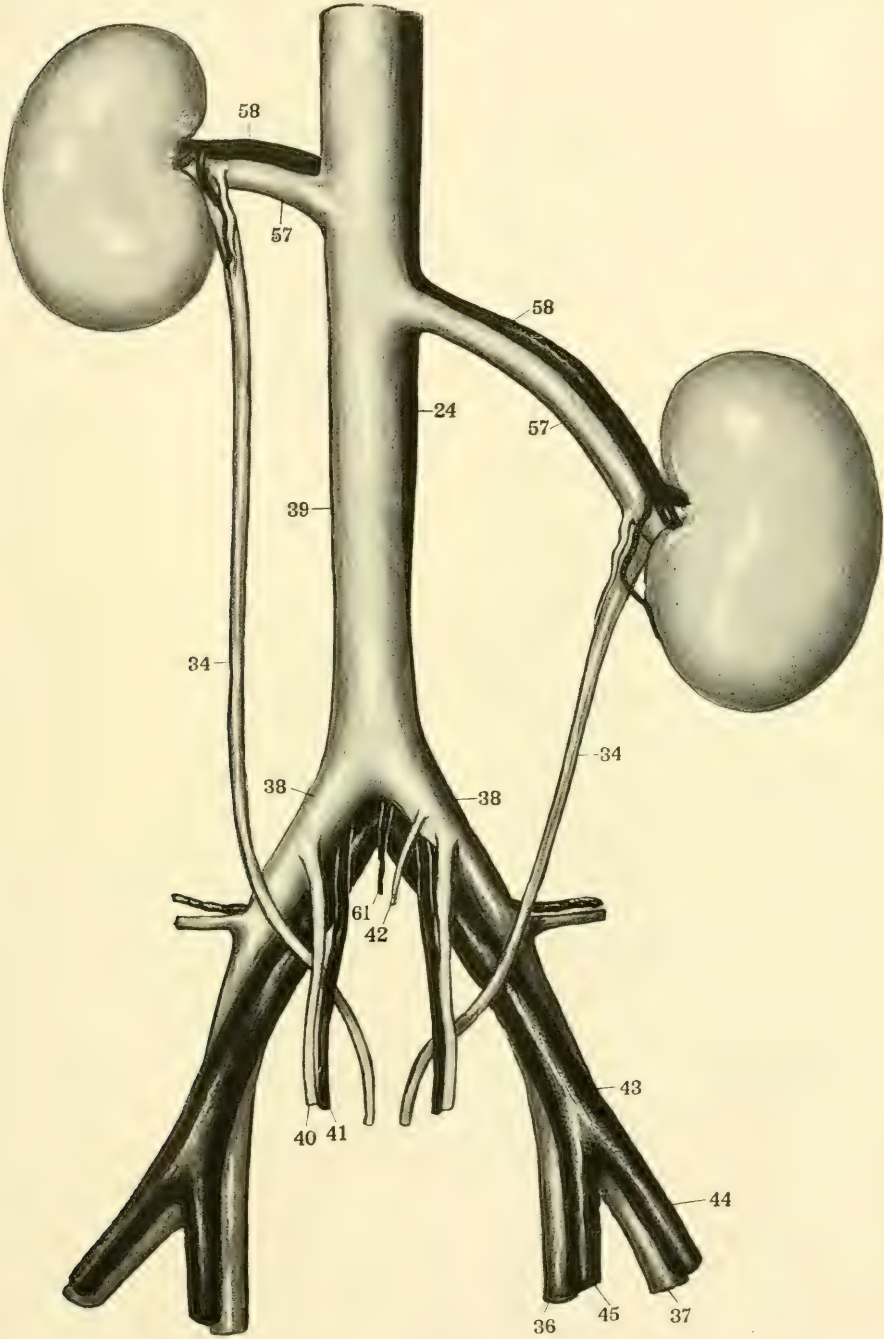


PLATE 2

EXPLANATION OF FIGURE

2 From a reconstruction of a 5 mm. *Tragul* embryo. Collection No. 204. $\times 100$. Showing the symmetrical arrangement of the axial venous channels and the plexuses in connection with them. 1, aortic arches; 2, pre-cardinal vein; 3, dorsal pre-cardinal tributaries; 4, dorsal aorta; 5, post-cardinal vein; 6, umbilical vein; 7, umbilico-post-cardinal plexus; 8, perimesonephroic plexus, 9, omphalomesenteric vein; 10, duct of Cuvier.

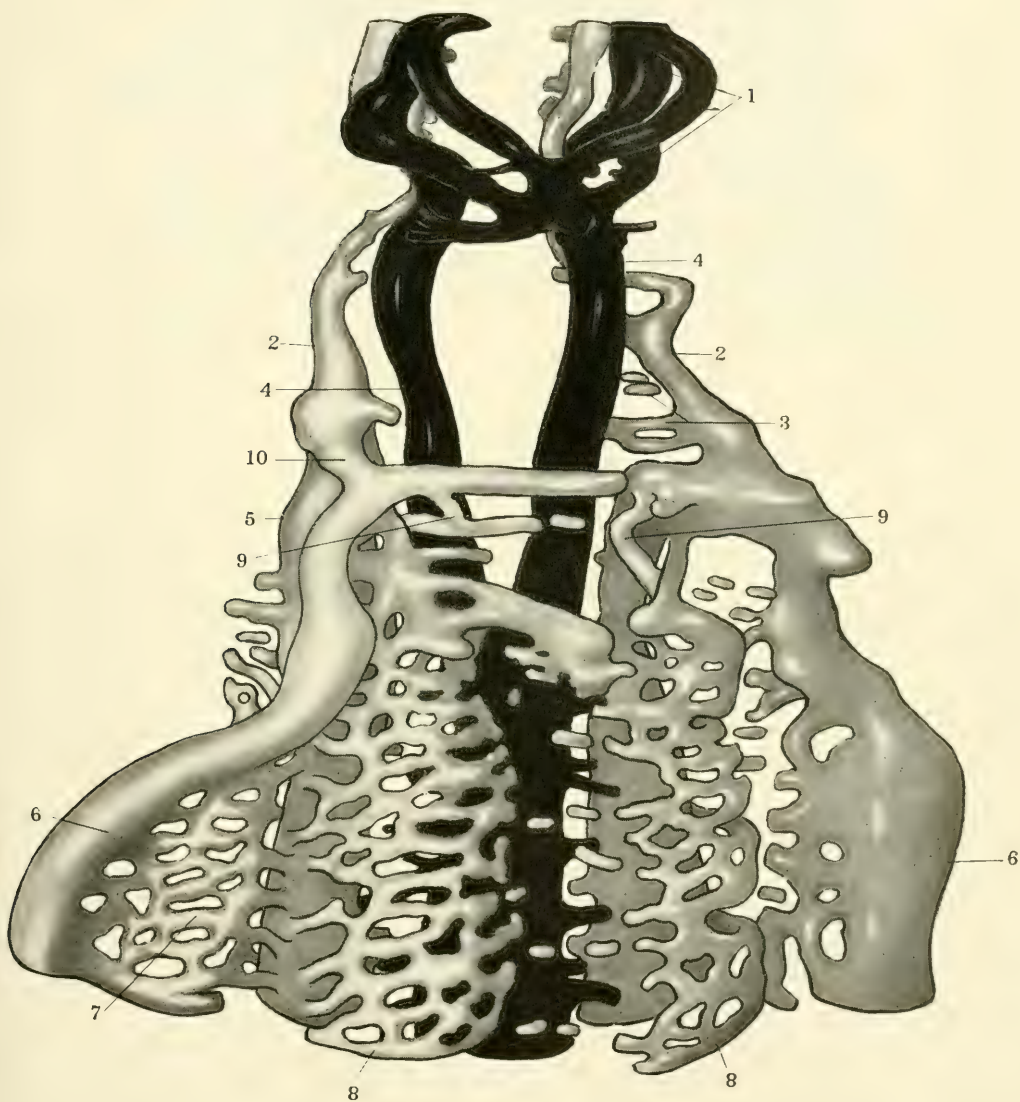
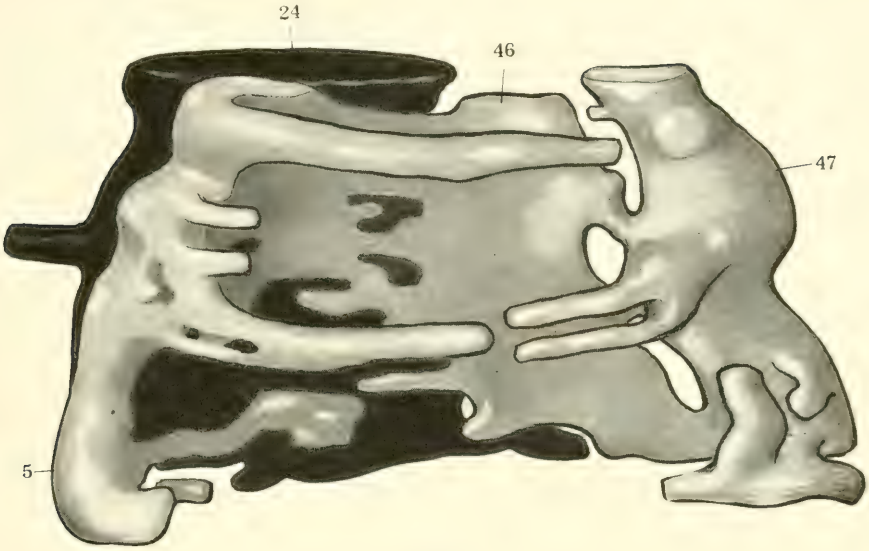


PLATE 3

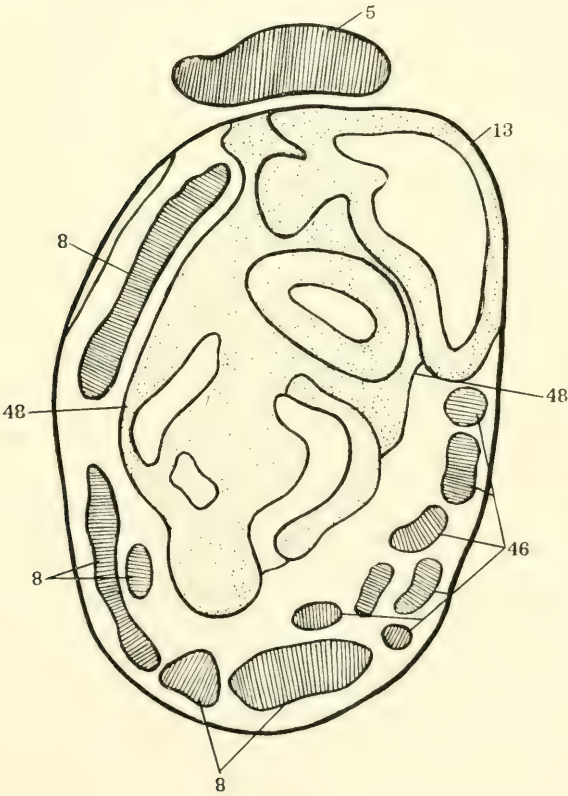
EXPLANATION OF FIGURES

3 Reconstruction of a portion of the perimesonephroic plexus in a 5 mm. *Tragulus* embryo. Collection No. 204. $\times 200$. 5, post-cardinal vein; 24, aorta; 46, mesial portion of perimesonephroic plexus; 47, ventral portion of perimesonephroic plexus.

4 Cross section showing the relations of the perimesonephroic plexus in a 5 mm. *Tragulus* embryo. Collection No. 204. $\times 200$. 5, post-cardinal vein; 8, perimesonephroic plexus; 13, Wolffian duct; 46, mesial portion of the perimesonephroic plexus; 48, mesonephros.



3



4

PLATE 4

EXPLANATION OF FIGURE

5 Reconstruction of a 6 mm. *Tragulus* embryo. Collection No. 205. $\times 100$. Showing the arrangement of the axial venous channels. 1, aortic arches; 2, pre-cardinal vein, showing its horizontal and vertical limbs; 3, dorsal pre-cardinal tributaries; 5, post-cardinal vein; 6, umbilical vein; 7, umbilical-post-cardinal plexus; 9, omphalomesenteric vein; 10, duet of Cuvier; 11, sub-hepatic sinus; 12, sub-cardinal vein; 13, Wolffian duet; 14, Cloaca; 24, aorta.

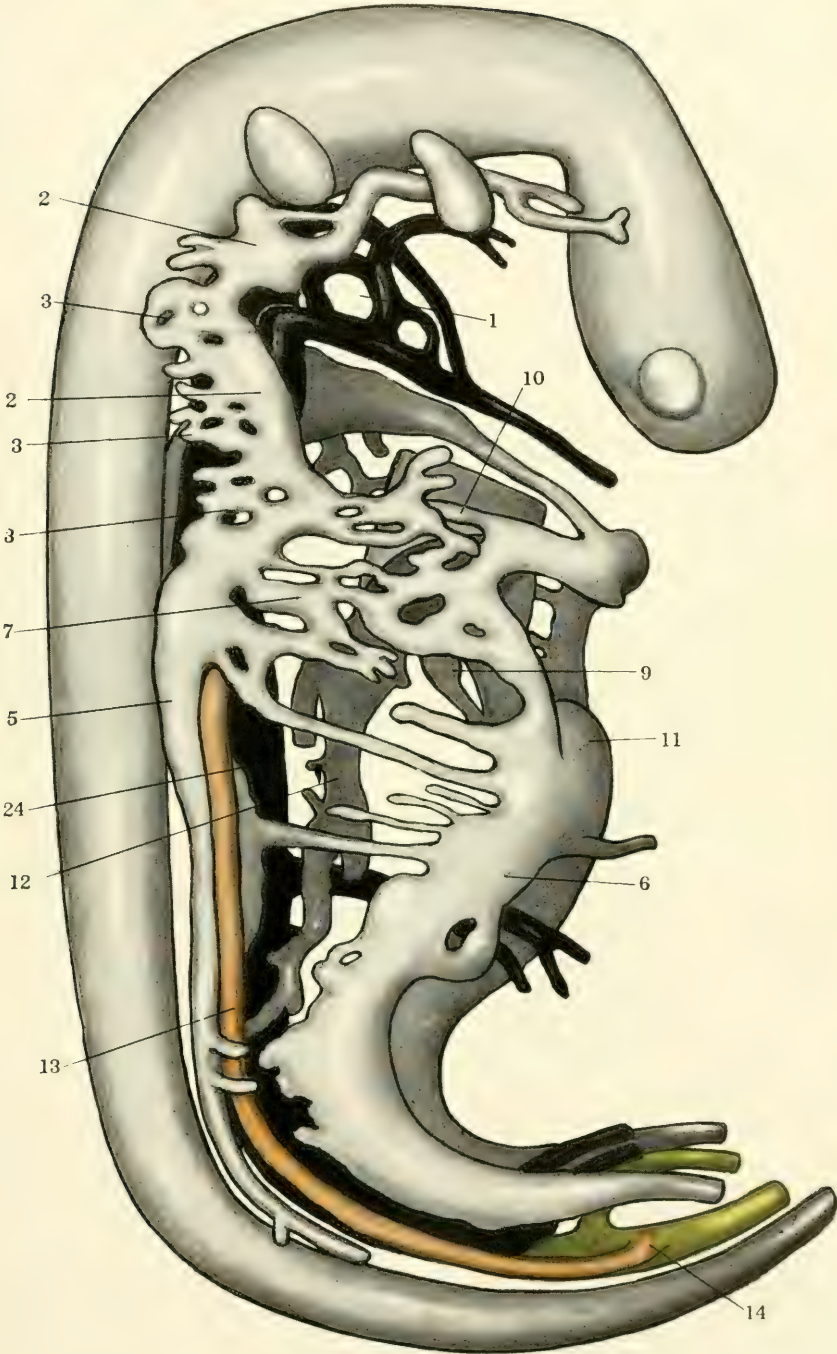


PLATE 5

EXPLANATION OF FIGURE

6 Reconstruction of a 6 mm. *Tragulus* embryo. Collection No. 205. $\times 100$. Showing the region of the sub-hepatic sinus in ventral view. 5, post-cardinal vein; 6, umbilical vein; 7, umbilico-post-cardinal plexus; 9, omphalo-mesenteric vein; 11, sub-hepatic sinus; 12, sub-cardinal vein; 13, Wolffian duct; 15, Sinus venosus; 16, intestine; 24, aorta.

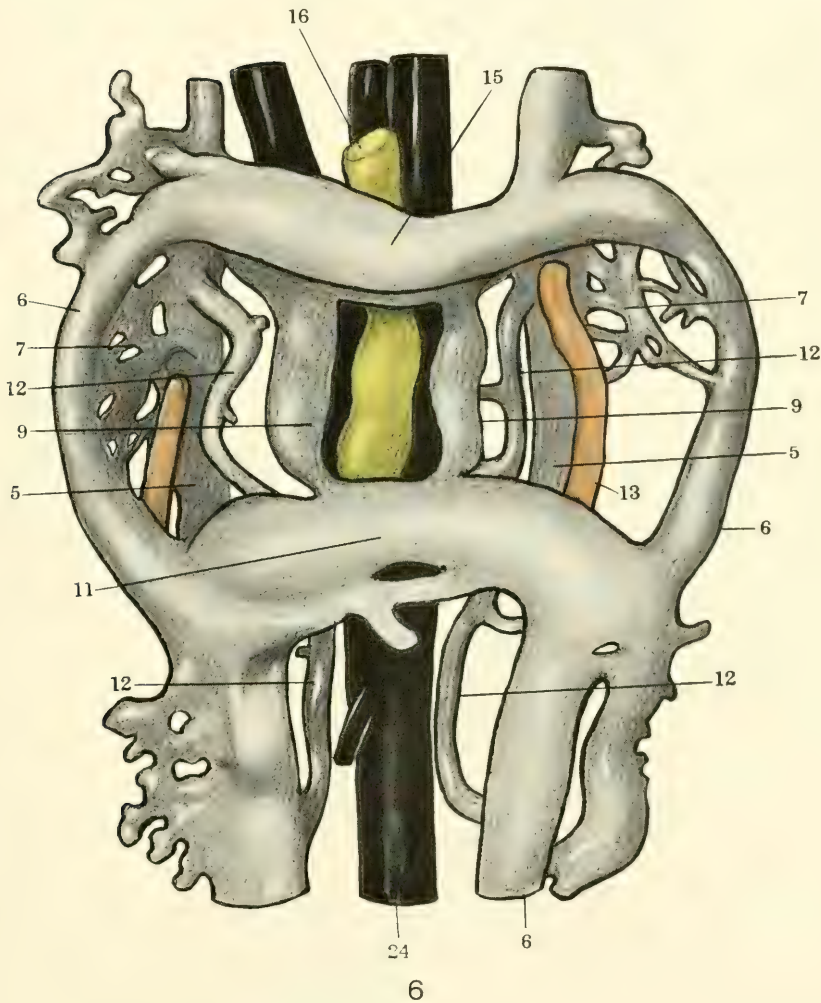


PLATE 6

EXPLANATION OF FIGURE

7 Reconstruction of a 20 mm. *Tragul* embryo. Collection No. 202. $\times 100$. Showing the axial veins and lymphatics. 6, umbilical vein; 17, pre-cava; 18, hepatic portion of post-cava; 19, sub-hepatic portion of post-cava; 20, post-cardino-cardinal collateral anastomosis; 21, cardinal collateral vein; 22, confluence of iliac veins; 23, arch of aorta; 24, aorta; 25, jugula lymph sac; 26, dorso-lateral process of the lymph sac; 26a, dorsal descending process of the lymph sac (thoracic duct approach); 26b, ventral descending process of the lymph sac (broncho-mediastinal approach); 28, azygos segment of thoracic duct; 29, post-azygos segment of thoracic duct; 30, cephalic division of pre-azygos segment of thoracic duct; 31, caudal division of pre-azygos segment of thoracic duct; 32, sympathetic nerve; 33, vagus nerve; 34, ureter; 35, jugular vein; 51, azygos vein; 62, dorsal segmental vein; 68, pancreas.

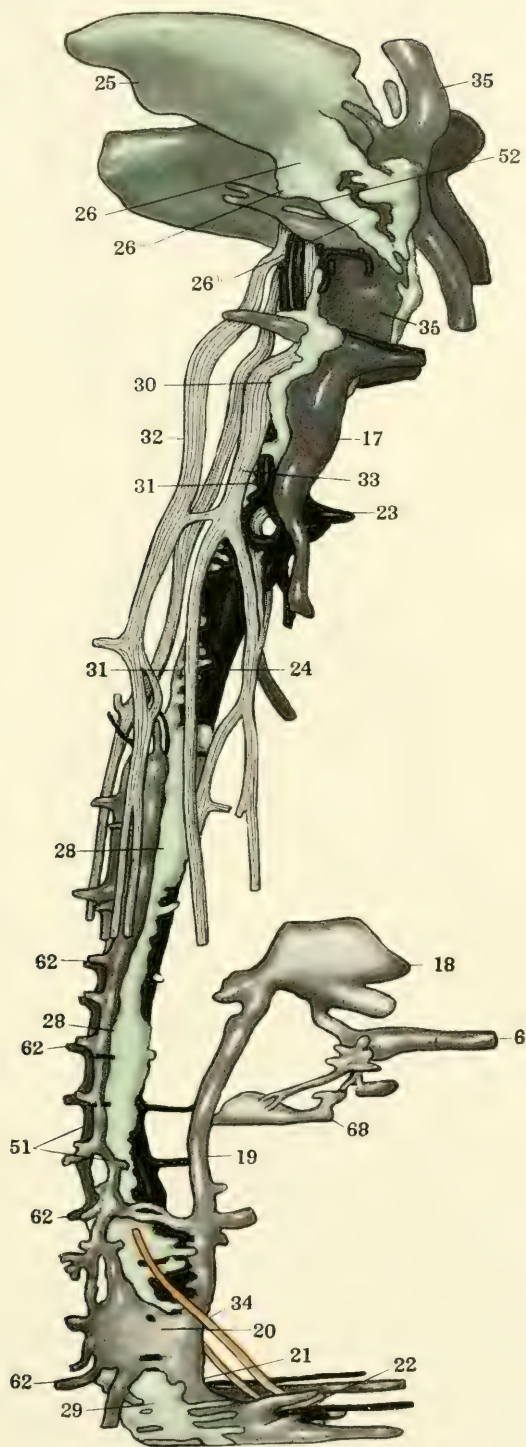


PLATE 7

EXPLANATION OF FIGURE

8 Reconstruction showing the junction of the azygos and pre-azygos segments of the thoracic duct in a 20 mm. *Tragulus* embryo. Collection No. 202. $\times 100$. 24, aorta; 28, azygos segment of the thoracic duct; 31, caudal division of the pre-azygos segment of the thoracic duct; 50, junction of the azygos and pre-azygos segments of the thoracic duct.



PLATE 8

EXPLANATION OF FIGURE

9 Cross section through the azygos segment of the thoracic duct showing its resemblance to the reptilian type of thoracic duct. 20 mm. *Tragulus* embryo. Collection No. 202. $\times 100$. 24, aorta; 28, azygos segment of thoracic duct completely surrounding aorta; 51, azygos vein.

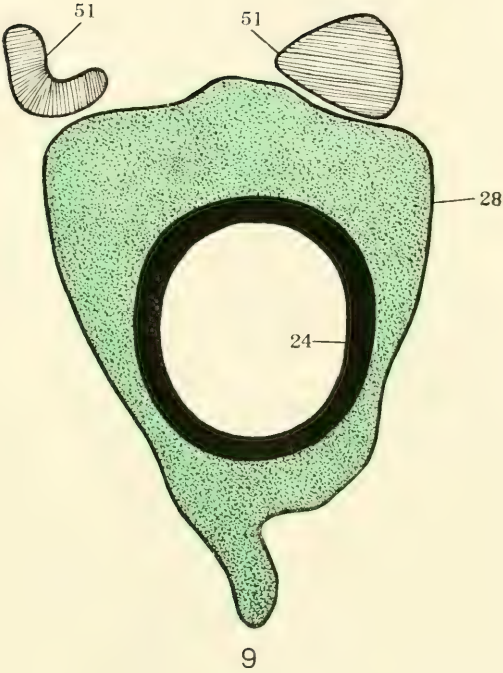


PLATE 9

EXPLANATION OF FIGURE

10 Reconstruction showing the axial veins and lymphatics in a 23 mm. *Tragulus* embryo. Collection No. 228. $\times 100$. 2, pre-cardinal vein; 23, arch of aorta; 25, jugular lymph sac; 26, dorso-lateral process of the lymph sac; 28, azygos segment of thoracic duct; 29, post-azygos segment of thoracic duct; 31, caudal division of the pre-azygos segment of the thoracic duct; 32, sympathetic nerve; 33, vagus nerve; 35, jugular vein; 51, azygos vein; 62, dorsal segmental vein; 65, sub-hepatic portion of post-cava.

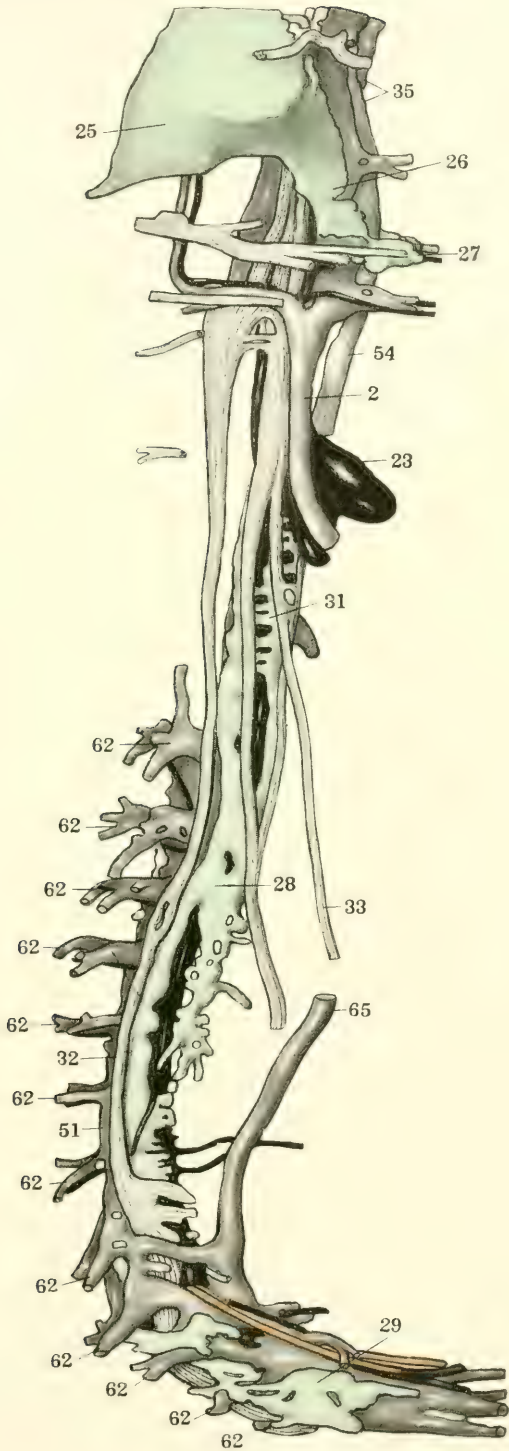
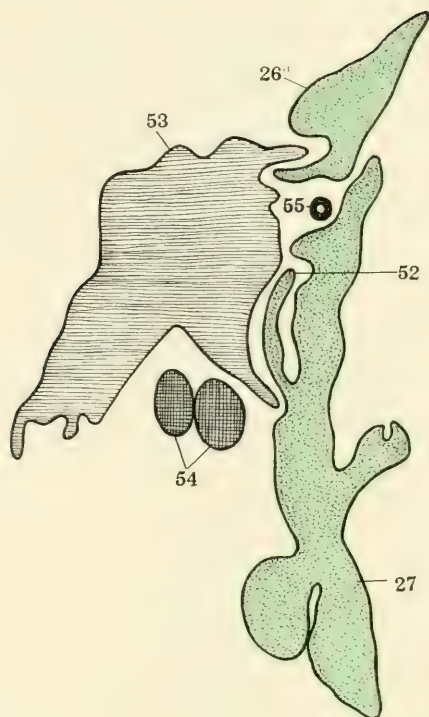


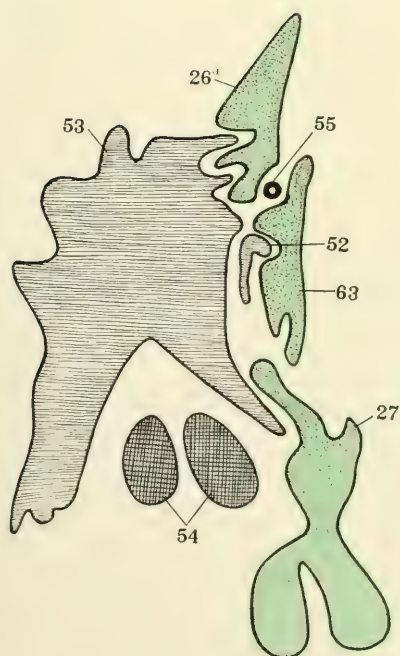
PLATE 10

EXPLANATION OF FIGURE

11 *A*, *B*, and *C*. Three serial sections showing the manner in which the left jugulo-sub-clavian tap of the lymph sac is made in a 23 mm. *Tragulus* embryo. Collection No. 228. $\times 100$. *26a*, dorsal descending process of the lymph sac (thoracic duct approach); *27*, ventral descending process of the lymph sac (broncho-mediastina approach); *52*, jugulo-sub-clavian approach; *53*, confluence of the jugular veins; *54*, thymus; *55*, thyro-cervical artery; *56*, sub-clavian vein; *63*, ventral prolongation of the jugulo-sub-clavian approach. The relations of the structure marked *52* in the three figures indicate the manner in which the secondary connection between the lymph sac and the venous system is made.

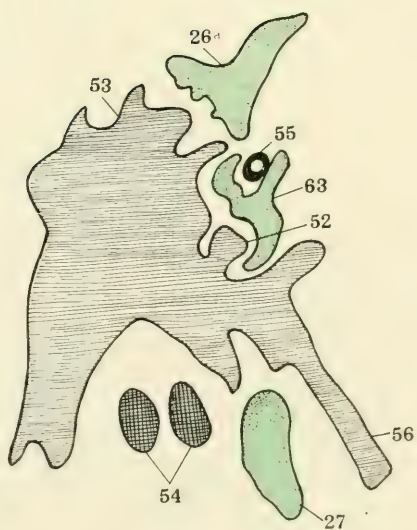


A



B

11



C

PLATE 11

EXPLANATION OF FIGURE

12 Reconstruction showing the junction of the azygos and pre-azygos segments of the thoracic duct in a 23 mm. *Tragulus* embryo. Collection No. 228. $\times 100$. 23, arch of aorta; 24, aorta; 28, azygos segment of the thoracic duct; 30, cephalic division of the pre-azygos segment of the thoracic duct; 31, caudal division of the pre-azygos segment of the thoracic duct; 30-31, confluence of the two divisions of the pre-azygos segment of the thoracic duct; 50, junction of the azygos and pre-azygos segments of the thoracic duct.

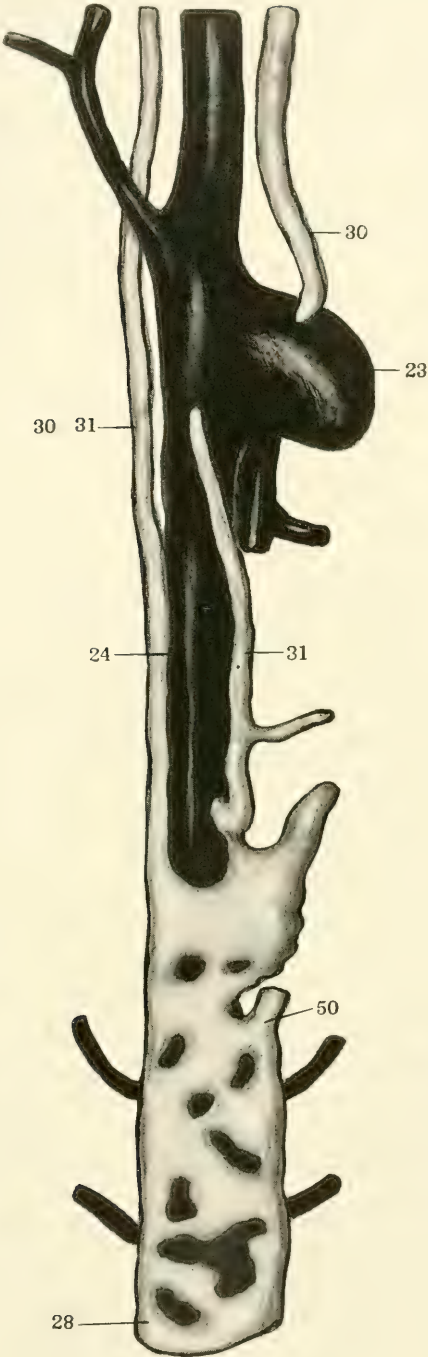


PLATE 12

EXPLANATION OF FIGURES

13a and 13b Schemata giving a ventral view of the relations of the post-cava to the aorta in a 20 mm. and a 23 mm. *Tragulus* embryo respectively, as shown by reconstructions of these stages. 21, cardinal collateral vein; 24, aorta; 40, sex vein; 41, spermatic artery; 57, renal vein; 59, inter-renal segment of the cava; 60, iliac bifurcation of the aorta.

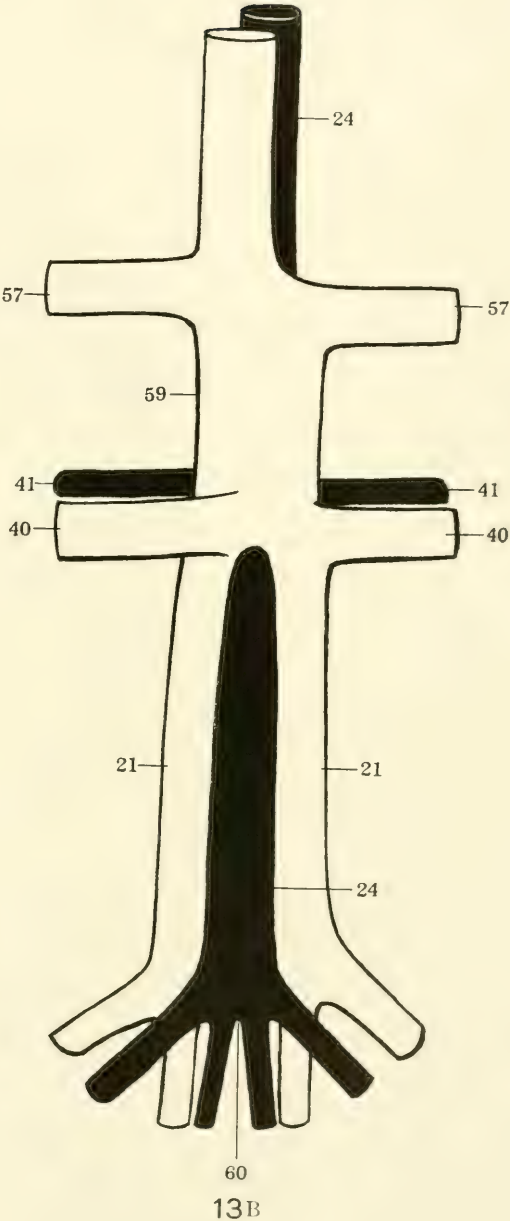
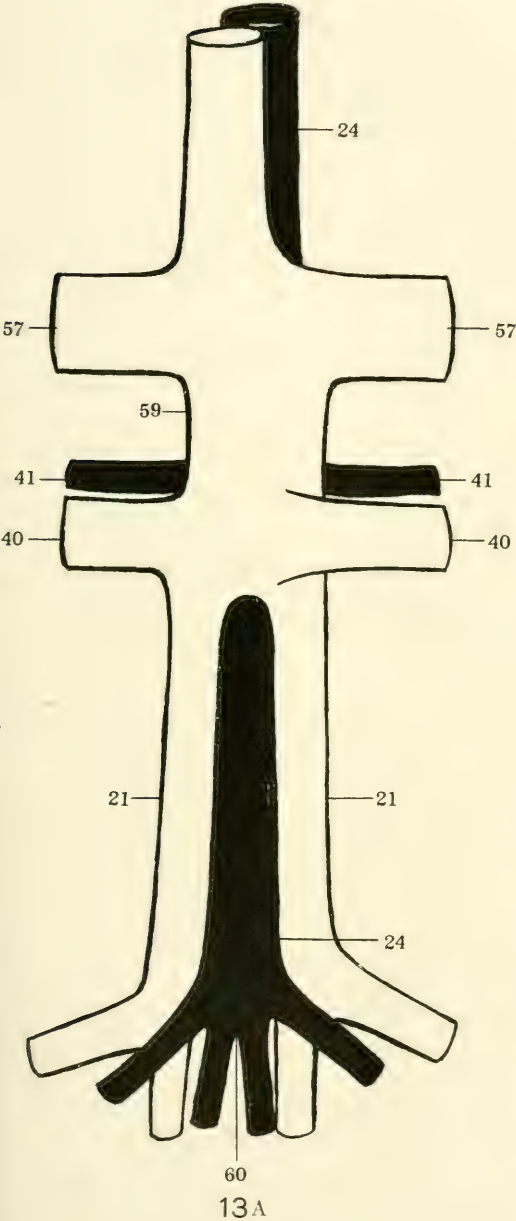
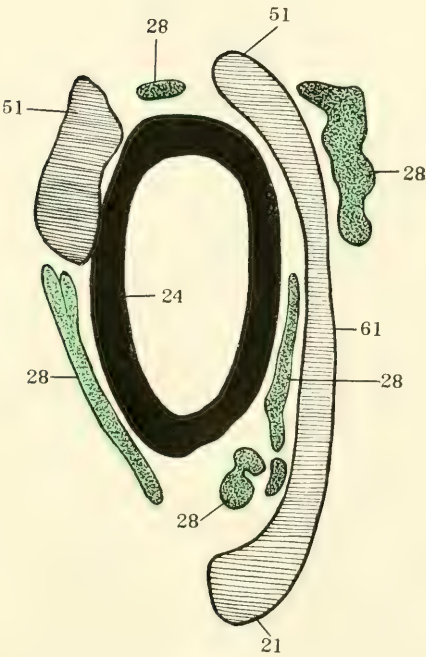


PLATE 13

EXPLANATION OF FIGURE

14 Cross section showing the anastomosis between the cardinal collateral and azygos veins in a 23 mm. *Tragulus* embryo. Collection No. 228. 21, cardinal collateral vein; 24, aorta; 28, azygos segment of the thoracic duct; 51, azygos vein; 61, anastomosis between cardinal collateral and azygos veins.



14

ON THE DEVELOPMENT OF THE HUMAN HEART

FRANKLIN P. MALL

From the Anatomical Laboratory of the Johns Hopkins University

THIRTY-SEVEN FIGURES

In my recent study¹ on the musculature of the adult human heart it was necessary to refer constantly to the development of this organ, and in general my description of the course of the muscle bundles was also based upon their development. This made it necessary to study numerous serial sections of embryo hearts, as well as whole hearts which had been removed from the embryo and dissected under the binocular microscope.

It is my purpose now to give as accurate a description as possible of several points which were obscure to me at the beginning of my study, so this report is to be viewed as supplementary to the excellent study by His as well as the recent one by Tandler. First of all an attempt was made to study the course of the muscle bundles and the formation of the vortex in the smallest hearts by means of direct observation upon whole hearts, stained and unstained, under the binocular microscope. This study was controlled by that of serial sections of other hearts, an abundance of material of both kinds being available. It soon became apparent that the muscle wall of the entire heart had to be included in this study, which soon showed that the critical point lay in the wall of the atrial canal, that is the common canal between the atria and ventricles. The study led back to the study of the valves at this point, an understanding of which is really the key to the whole situation. This resulted in locating definitely the atrio-ventricular muscle bundle (bundle of His) in all stages of development.

¹ Mall, F. P., On the muscular architecture of the ventricles of the human heart. Amer. Jour. Anat., vol. 11, 1911.

The points to be discussed will be considered in the following order: A, Subdivisions of the early heart; B, Formation of the septum and atrio-ventricular valves; C, The atrio-ventricular bundle; D, Musculature of the left ventricle.

A. SUBDIVISIONS OF THE PRIMARY HEART TUBE

In an embryo about 2 mm. long (No. 391), which was modeled in wax by Dr. Dandy, the heart is shown as a relatively straight tube with its arterial end directed towards the head (fig. 1). Its muscle wall is of even thickness and communicates throughout its whole length along the dorsal midline with the rest of the mesoderm, that is it has a complete dorsal mesentery.² At its anterior end the heart tube shows a slight dilatation just before the arteries arise from it. Midway between the two ends of the muscle tube there is an indentation on the left side which marks the beginning of the bulbo-ventricular groove, that is, it separates the atria and left ventricle on the one hand from the right ventricle and the bulb on the other. Within the muscle tube there is suspended by means of numerous fine fibrils the collapsed endocardial lining. These fibrils will be considered later when the development of the valves is discussed.

The heart now separates rapidly from the rest of the mesoderm in subsequent stages and is soon suspended in the pericardial coelom, remaining attached to the body only at its venous and arterial ends. The indentation on the left side of the heart, mentioned above, becomes more pronounced, the heart rolls upon itself quite rapidly as succeeding stages of development show. In an embryo 2.5 mm. long (No. 3) the heart is separated from the dorsal midline in its middle, while in another of the same length (318) the separation is more pronounced. In one 3.5 mm. long (164) the separation is complete and the various subdivisions of the final heart tube can be outlined with precision (fig. 2).

² A complete description of this embryo is given by Dandy. *Amer. Jour. Anat.*, vol. 10, 1910. Sections through the heart are shown in his figs. 1 and 2. Evans (Keibel-Mall, *Manual of human embryology*, vol. 2, fig. 409) also pictures sections through the heart of this embryo. Also by Mall, *Ibid.*, vol. 1, figs. 382-386.

LEGENDS FOR ALL OF THE FIGURES

A, atrium
A.C., atrial canal
A.Cu., anterior endocardial cushion
A.F., annulus fibrosus
Ao., aorta
A.P., anterior papillary muscle
B.S., bulbo-spiral band
A.V.B., atrio-ventricular bundle
B., bulb
C.S., coronary sinus
I.V.C., interventricular canal
*F.O.*¹ foramen ovale I
*F.O.*² foramen ovale II
L.A., left atrium
L.Cu., left endocardial cushion
L.O., left venous ostium

L.P., large papillary muscle of the right ventricle
L.R.V., longitudinal bundle of the right ventricle
L.V., left ventricle
M.p.m., medial papillary muscle
P., pulmonary artery
P.Cu., posterior endocardial cushion
P.P., posterior papillary muscle
R.A., right atrium
R.Cu., right endocardial cushion
R.O., right venous ostium
R.V., right ventricle
S.A., septum of the atria
S.S., sino-spiral band
S.V., septum of the ventricle

V., ventricle

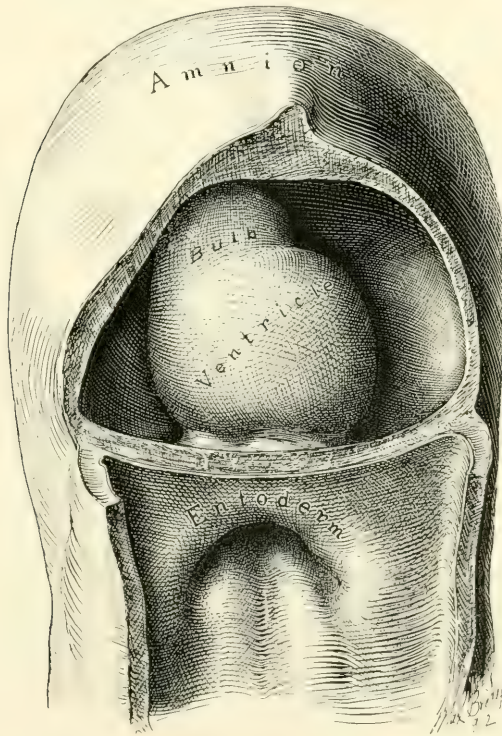


Fig. 1 Ventral view of the model of an embryo 2 mm. long (No. 391). $\times 100$. The pericardium has been removed from in front of the heart.

I mentioned above in describing the heart of the embryo 2 mm. long that its endothelial lining is collapsed and suspended by a mass of fine fibrils within the muscular tube. In slightly older stages the arrangement of the endothelial lining is changing, becoming dilated on the venous side of the heart. This change is beginning in No. 3, is more advanced in No. 318 and is complete in No. 164 (fig. 2). His³ has pointed out that the endothelial lining hugs the muscle wall closely in the embryonic atrium, while it remains suspended for a time in the rest of the heart. This arrangement is so pronounced in the early heart that it affords a way by which

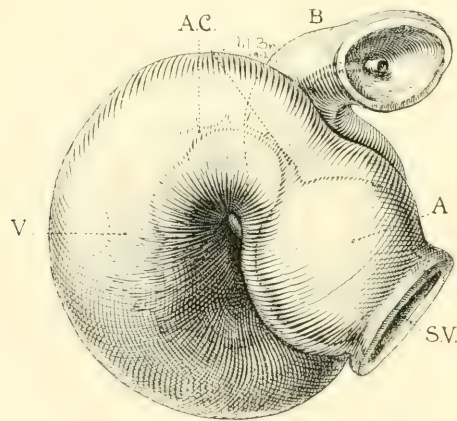


Fig. 2 From the reconstruction of a heart of an embryo 3.5 mm. long (No. 164). $\times 66$. View from the left side.

we may determine with precision the exact portion of the heart tube from which the atrium arises. My specimens show conclusively that the atrium arises exclusively from the free heart tube and that the sinus venosus does not contribute to its formation. This being established it follows that as soon as the heart tube is fully separated from the body walls that the anlage of the entire adult heart is to be found between its arterial and venous attachments.

In the embryo 3.5 mm. long (No. 164) the completed heart tube is seen, which is S-shaped and twisted upon itself so that the

³ His, W., *Anatomie mensch. Embryonen*. Theil 3, Leipzig, 1885.

arterial and venous ends are brought close together. At the venous end the muscle wall is slightly dilated which marks the atrium; this is lined closely with endothelium which encircles the cavity within. No delicate fibrils are here seen between the muscle wall and its endothelial lining. Then follows an upper bend to the heart after which there is a dilatation projecting towards the left side, the former marking the atrial canal and the latter the left ventricle. The lower connecting piece unites the left ventricle with the bulb which later on gives rise to the right ventricle. In the atrial canal (Haller's auricular canal) the endothelial tube is seen as a solid strand of cells suspended freely in the muscle wall

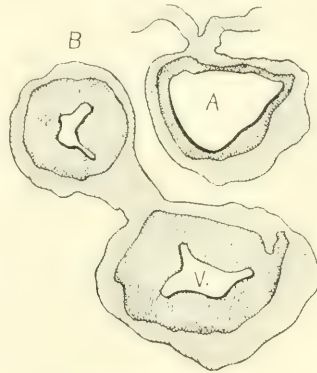


Fig. 3 Section of the heart of the embryo 3.5 mm. long. $\times 66$.

by the delicate fibrils already mentioned. In the left ventricle the tube shows a distinct cavity, while throughout the rest of the heart tube the cavity is irregular but not pronounced. The form of the endothelial tube is shown in fig. 2 and again in a semidiagrammatic figure of a transverse section through the atrium, ventricle and bulb in fig. 3. The delicate fibrils, which no doubt belong to the endothelial cells are present in large number throughout the whole heart tube, excepting in that which forms the atrium. In another embryo (No. 384, 2 mm. long), considerably smaller than the one just described and probably pathological, the degree of development of the heart is practically identical with the one $3\frac{1}{2}$ mm. long (No. 164).

In my collection there are two other embryos slightly more advanced in development than the one just described which bear upon the exact origin of the atria from the heart tube. They are Nos. 486 (4 mm. long) and 470 (4 mm. long). Neither of these specimens has been studied carefully as a whole, so the number of myotomes in each can not be given. Nor have the measurements been corrected by the drawings and the sections.

In No. 486 the single atrium as described above (No. 164) is more pronounced, is dilated and filled with blood, while the form of the endothelial tube is much the same. However, the atrium is sharply separated from the sinus venosus and there are a few fibrillae between the endothelial and muscular walls. In the left ventricle the endothelial and the muscular layers are just beginning to interlock to form the first trabeculae.

Embryo No. 470 shows the heart more advanced than in No. 486. The atrium has become double, that is there are two atria. The endothelial tube in it is distended and its separation from the sinus venosus is still pronounced. The trabecular formation in the left ventricle is somewhat more pronounced than before.

From now on the changes in the heart take place very rapidly, as the general form of the embryo also changes rapidly. The head is bent upon the body which is well curved upon itself with pronounced limb buds. In the next embryo, No. 239 ($4\frac{1}{4}$ mm. long), the subdivisions of the heart are sharply defined and in the following stage, No. 463 (3.9 mm.), the preliminary subdivisions are complete (fig. 4). During this time the embryo curls upon itself and the limb buds are formed. In embryo 239 the two atria are very pronounced, the right communicating with the sinus venosus. The atrial canal is sharply defined, first as a constriction and secondly by a great increase of the fibrillar mass between the endothelial and muscular walls. The trabecular system is well formed in the left ventricle and has extended into the bulbus, that is into the right ventricle. In this specimen it is clear that the course of the circulation is from the right to the left atrium, then first to the left ventricle after which it enters the right. The right atrium still lies in the notch between the left ventricle and the bulb on the posterior side of the heart. Only a little later

does it project to the right side of the bulb which becomes its permanent position. This is seen in No. 463 (fig. 4).

Although the two embryos just mentioned are practically of the same stage of development, the difference of the degree of development of their hearts is most pronounced. No. 463, which is perfectly preserved, has a heart with larger atria, a more constricted atrial canal, a large left ventricle and a pronounced but contracted bulb. The muscular wall of the whole heart is continuous without a single break in it. That surrounding the atrial canal is sharply defined forming a continuous ring connecting at

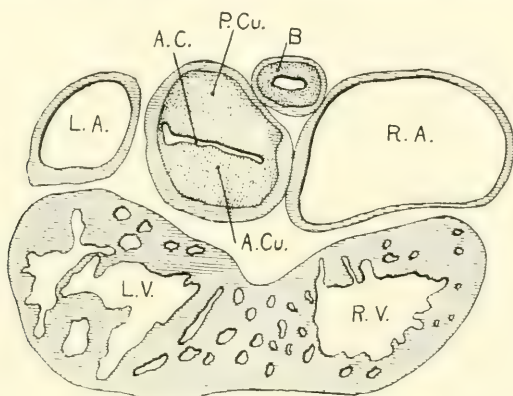


Fig. 4 Section of the heart of an embryo 3.9 mm. long (No. 463). $\times 66$.

all points with the atria above and the left ventricle below. This is mentioned especially because a share of this connecting ring disappears while the remaining portion becomes the atrio-ventricular bundle.⁴

B. FORMATION OF THE ATRIO-VENTRICULAR VALVES

In the earliest stages described (No. 391), while the muscle wall of the heart is still in the form of a straight tube and is connected throughout its length with the body wall, the endothelial tube is separated from the muscular tube by a marked layer of delicate fibrils. In their papers upon the heart both His and

⁴ A few data regarding all of the embryos described in this paper are given in my catalogue of 500 specimens. *Anat. Rec.*, vol. 5, 1911.

Tandler speak of these fibrils but they give no very definite information regarding their nature. In his study of the chick His⁵ describes the space between the endothelial tube and muscular wall of the heart, which later in development fills with connective tissue arising from the inner tube. In the atria, where this space is never pronounced, the secondary thickening is also insignificant. Later His⁶ also observes this space in young human embryos. Many fibrils extend from the endothelial tube, which when they are pronounced, draw out the side of the tube in a characteristic way. This he pictures. He is uncertain whether these fibrils are natural or produced by the hardening reagents used. In the atrium the inner tube hugs the muscle wall closely. In the atrial canal the space is filled with two pronounced cushions of connective tissue, while in the ventricle the muscle forms trabeculae which are soon covered with endothelium. In the bulb this space is very marked and filled with a delicate connective tissue framework. He does not show conclusively the meaning of this tissue.

Tandler⁷ describes and figures this substance well in a human embryo with fifteen somites. Although his figure shows beautifully the inwandering of nuclei from the endothelium, and although he speaks of a reduction and enlargement of these fibrils, he is unwilling to decide whether or not they are due to the method of preservation and of staining of the sections. He states expressly that the tissue resembles very much Wharton's jelly. It seems to me, however, that the evidence of His and Tandler is sufficient to show that this tissue is not due to coagulation but a constant normal constituent of the developing heart. That it is distributed in a definite way in different portions of the heart and in different stages of development speaks almost conclusively for this opinion. Its origin and meaning is however a different question.

⁵ His, *Untersuch ueber die erste Anlage des Wirbelthierleibes*. Leipzig, 1868, S. 141.

⁶ His, *Anat. mensch. Embryonen*. Th. 3, 1885, S. 141.

⁷ Tandler, *Entwicklungsgeschichte des Herzens*. Keibel-Mall *Handbuch d. Entwickl. d. Menschen*, Leipzig, 1911, Bd. 2, S. 524.

In the youngest embryos studied the reticular mass between the endothelial cells and the muscle wall appears either homogeneous, or as composed of most delicate fibrils, or of coarser fibers, according to the method of preservation. In general it appears like the most delicate reticulum of the mesenchyme and under all circumstances any stain which tinges the fibrils tinges also the endothelial cells. So intimate is this connection that it forces the conclusion that the fibrils together with the endothelial cells form a syncytium. In the younger hearts the endothelial nuclei lie altogether on the inner side of the fibrils as has been repeatedly observed, but as soon as the trabecular system begins to form in the left ventricle some of the endothelial nuclei invade the fibrillar layer. This is first seen in embryo No. 239 ($4\frac{1}{4}$ mm. long). Here the trabecular system is quite completely formed in the left ventricle by an interlocking of processes from the muscular and endothelial layers. In the right ventricle the process is not so far advanced, while in the atrial canal and the bulb the reticular layer is invaded by endothelial nuclei but not by muscle cells. A similar arrangement is found in embryo No. 3, which as No. 239, is intensely stained. Fig. 5 is from the posterior endocardial cushion of an embryo 4.3 mm. long, (No. 148), showing that the nuclei of the cushion are invading it from its endothelial side. All this is more pronounced in No. 463 (3.9 mm.) which is more advanced in development and is perfectly preserved. In this specimen it is quite easy to demonstrate that the nuclei of the endothelium and the reticular mass belong together, for they are distinctly intermingled and yet are separated from the muscular layer (fig. 6). Since the nuclei and fibrils belong together and since it has been demonstrated that the reticulum of the liver is developed from the endothelial cells, I shall speak of the reticulum between the endothelium and muscle layer of the heart as endothelial fibrils. The great importance of this distinction is at once apparent for it shows that connective tissue arises also from endothelial cells and that the intima of the entire vascular system including the the valves of the heart has a like origin.

I think that I have now shown that the endothelial fibrils are constant in the heart and that we must hold the endothelial

cells responsible for the production of the connective tissue of the endocardium as well as of the valves. Further study will probably show that endothelial connective tissue is by no means of rare occurrence. At any rate it has been definitely settled that the endothelial cells of the liver give rise to the connective tissue of the liver lobule.

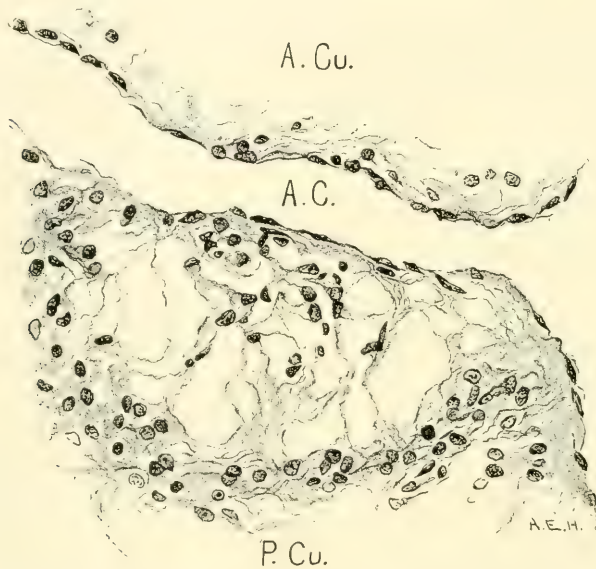


Fig. 5 Section of the posterior endocardial cushion of an embryo 4.3 mm. long (No. 148). $\times 360$.

In my study on the development of the connective tissue I was astonished to find in macerated and digested frozen sections that the endothelial tube with its surrounding reticulum can be isolated.⁸ In such specimens it is impossible to separate the nuclei from this continuous mass of reticulum; together they form a syncytium. This connection was demonstrated in pig embryos 20 mm. long. Although this was entirely out of harmony with the results obtained for other connective tissues, which always arise from the mesenchyme, it had to be accepted and so it was recorded.

⁸ Mall, Development of the connective tissues from the connective syncytium Amer. Jour. Anat., vol. 1, 1902, p. 354.

In a measure this was confirmed by Kon⁹ who observed the development of the reticulum in the liver of a foetus in the middle of pregnancy. Mollier¹⁰ in his beautiful study on the development of the blood shows conclusively the connection between the endo-



Fig. 6 Section of the anterior endocardial cushion in the atrium of an embryo 3.9 mm. long (No. 463). $\times 360$.

thelial cells of the liver and the surrounding reticulum. This he follows back to a human embryo 10 mm. long and in subsequent stages the connection of the endothelial with the reticulum is

⁹ Kon, Die Gitterfasergerüst der Leber, etc. Archiv. für Entwickl.-Mechanik, Bd. 25, 1908.

¹⁰ Mollier, Die Blutbildung in der embryonalen Leber des Menschen. Archiv für mik. Anat., Bd. 74, 1909.

complete, that is, it forms a syncytium. Although Mollier believes that the capillaries of the liver arise from the mesenchyme of the capsule, which is impossible, it answers our purpose to state that he shows that the connective tissue of the liver develops from the endothelial cells and not from other mesenchyme cells. Those of us who see the primary vascular tree of the liver arising from the endothelial wall of the omphalomesenteric veins by a process of reduction of this large vessel to form sinusoids, recognize Mollier's "origin" of capillaries of the liver as only a secondary contact between the sprouting capillaries when they reach the mesenchyme of the capsule of the anlage of the liver. For the present purpose it is clear that Mollier demonstrates also that the connective tissue of the liver arises from endothelial cells. This relationship has been amply confirmed by F. T. Lewis in the liver of a human embryo 7.5 mm. long.¹¹ So for the liver the chain is complete; throughout development the connective tissue of the lobule is in direct continuity with the endothelial cells of the blood capillaries and therefore they give rise to them. Within the lobule there are only endothelial cells and epithelial cells and no one finds the reticulum arising from the latter.¹²

In embryo No. 239 the endothelial fibrils are quite unequally distributed throughout the heart. In the atria, as mentioned above, they form but a very thin layer. In the atrial canal the

¹¹ Lewis, F. T., *Entwicklung der Leber*. Keibel-Mall, *Handbuch der Entwickl.*, Bd. 2, 1911. S. 397, fig. 291.

¹² Quite recently Mollier finds the endothelial cells and reticulum of the spleen as one continuous reticulum from which he concludes that the endothelial cells arise directly from the mesenchyme (*Arch. f. mik. Anat.*, Bd. 76, 1911). The opposite conclusion may be drawn equally well. I have published figures which correspond with Mollier's, giving at the same time a conclusive experiment to prove that the circulation of the spleen is entirely through the pulp spaces; these have the value of blood capillaries (Mall, *Amer. Jour. Anat.*, vol. 2, p. 315). Pathologists have been of the opinion that in endarteritis the intima thickens by a proliferation of endothelial cells, and that connective tissue may arise from these cells. Marchand has questioned the truth of this statement, but recently the subject has been reinvestigated by Baumgarten (*Arbeiten auf dem Gebeite der Pathologischen Anatomie*, Leipzig, 1904, Bd. 4) who showed that proliferation of endothelial cells may form a thickening of the intima without any rupture of the elastica interna, thus excluding entirely any participation of connective tissue cells in the process. See also von Szily, *Anatom. Hefte* 35, 1908, Taf. 45-47.

fibrils are heaped up into two mounds to form the well known endocardial cushions, which have between them a transverse slit. The posterior cushion extends upward into the left atrium and then along its posterior surface into the right atrium and ends at the opening of the sinus venosus. The anterior cushion also extends into the left atrium along its anterior border and reaches to the septum primum which is just beginning to form. Below, in the left ventricle, the endocardial cushions blend with the endothelial reticulum covering the trabeculae. The interlacement of the endothelial and muscular layers to form the trabeculae extends into the right ventricle, but in the bulb the two layers are quite sharply defined and separated.

The nuclei of the endothelial syncytium form first of all the inner layer of the heart, but in the endocardial cushions of the atrial canal as well as in the bulb the nuclei gradually extend towards the muscular coat. In other words the nuclei of the inner coat are gradually invading their reticular layer.

In the heart of embryo 463 the differentiation of the endothelial syncytium is more pronounced than in the specimen just described. The heart is now well formed with two pronounced atria, a much constricted atrial canal and a marked constriction of the interventricular canal (fig. 4). The bulbo-ventricular and the interventricular grooves are well formed. The septum of the atria and that of the ventricles are well marked. The endothelial syncytium is most pronounced in the endocardial cushions and in the bulbus. The cells are quite equally distributed throughout the syncytium but they are somewhat more numerous immediately under the endothelial covering than near the muscle layer of the heart (fig. 6). The posterior cushion does not reach as far into the left ventricle as the anterior and is also less extensive in the atria; it reaches nearly to the sinus venosus. The anterior endocardial cushion is a large sickle-shaped affair, encircles the heart in front as the border of the atrial septum (septum primum) which is now forming. The anterior cushion ends on the medial side of the opening from the sinus venosus. The space in the atria between the cushions marks the primary foramen ovale (foramen ovale I).

In an embryo 4.3 mm. long (No. 148) practically the same conditions are seen as in the embryo just described. If anything it is a little more advanced in development. A section of the endocardial cushion is shown in fig. 5. From now on new conditions arise which when concluded separate the heart into its right and left halves.

The anterior and posterior cushions are now well formed, the superior septum (primum) and the septum of the ventricles (septum inferior) are beginning but the septum aorto-pulmonale (aortic septum) is still absent. While these are forming, up to the next stage, when the muscle wall of the atrial canal begins to break down, the heart gradually enlarges without changing very markedly its external form. The steps which I am about to describe are well established in various mammals, but I shall repeat them hastily in order to confirm them all in the human heart. In doing this I shall include in the descriptions the following specimens:

- Embryo No. 80, C. R. length 5 mm.
- Embryo No. 136, C. R. length 4 mm.
- Embryo No. 116, C. R. length 5 mm.
- Embryo No. 241, C. R. length 6 mm.
- Embryo No. 2, C. R. length 7 mm.
- Embryo No. 383, C. R. length 7 mm.
- Embryo No. 380, C. R. length 7.5 mm.
- Embryo No. 113, C. R. length 8 mm.
- Embryo No. 397, C. R. length 8 mm.
- Embryo No. 422, C. R. length 9 mm.
- Embryo No. 163, C. R. length 9 mm.

In Embryo No. 80 the anterior and posterior cushions are considerably thicker than before but hold practically the same relation to the heart as in Nos. 463 and 148. In the left ventricle the cushions are spread out and have attached themselves to the trabecular system. As there are two attachments which correspond in position to the anterior and posterior papillary muscles, it is proper to speak of them as such. The septum aorto-pul-

monale is well formed and its two ridges reach well into the bulbus to the interventricular foramen. Much the same arrangement is found in Embryos No. 136, 116 and 380 which are of the same stage of development as No. 80.

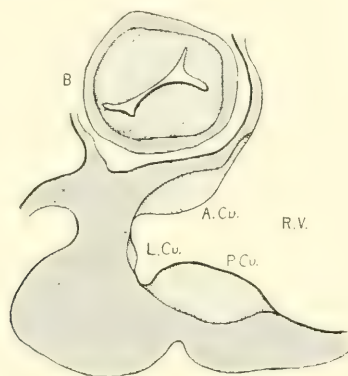


Fig. 7 Transverse section of the atrial canal and bulb of the heart of an embryo 9 mm. long (No. 422). $\times 40$.

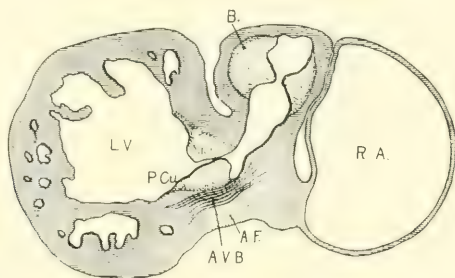


Fig. 8 Transverse section of the heart of an embryo 7 mm. long (No. 2). $\times 40$.

In Nos. 241, 422 and 2 the lumen of the bulb is ∞ -shaped, the endocardial cushions are much more pronounced than before; they are ready to fuse as is indicated in figs. 7 and 8. The septum primum and the interventricular septum are well marked. In 397 the septum primum is very thin above so that it is uncertain whether or not it has broken through to form the foramen ovale II.

In 383, 113 and 163 the foramen ovale II has just formed, being smallest in the first and largest in the last. The cushions are well developed in No. 383; the anterior reaches to the septum primum and the posterior to the opening of the sinus into the right atrium. There is a large space between them connecting the two atria. High up in the atria the septum primum is broken though forming the foramen ovale II, as shown by Born. The arrangement of the endocardial cushions with the space between them and the

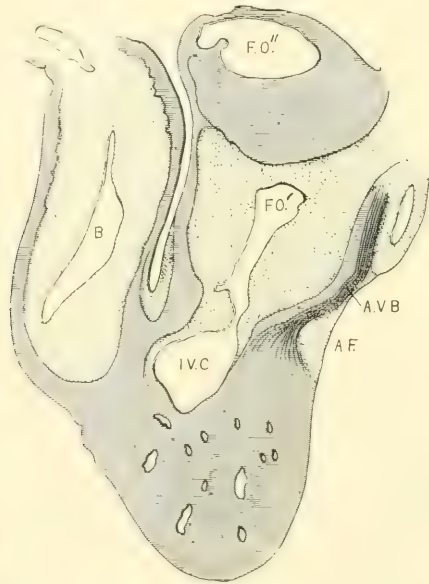


Fig. 9 Sagittal section. Embryo 8 mm. long (No. 113). $\times 40$.

foramen ovale II is well shown in No. 113 (fig. 9). Both cushions now course to the sinus venosus and are blended with the connective tissue above it. Behind the cushions there is a muscle strand from the sinus to the ventricle which marks the position of the atrio-ventricular bundle. The thin septum primum reaches to the two cushions, and high up it is perforated by an opening with sharply defined walls. Between the anterior and posterior cushions well within the atrial canal the two atria still communicate with each other; in this region the cushions are as yet not

blended. In No. 163 the blending of the cushions is complete (fig. 10) and the permanent foramen ovale is fully established. Together the united endocardial cushions form a cubical plug which blocks the center of the atrial canal leaving a channel on either side. It also projects into the left ventricle and is attached to its walls forming the two papillary muscles. The atrial canal is divided into two canals which now form the right and left ostia. The two cushions give rise to the medial cusps of the bicuspid and tricuspid valves, that is, the medial cusp of the tricuspid valve and the anterior cusp (B.N.A.) of the mitral valve; the right halves of each cushion make the former and the left halves the latter.

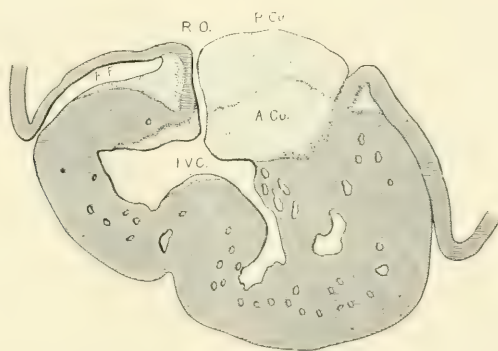


Fig. 10 Coronal section. Embryo 9 mm. long (No. 163). $\times 40$.

The septum aorto pulmonale is still incomplete in the specimens described, and the interventricular foramen is wide open, but the septum of the ventricle is well formed, extends upward and blends with the posterior endocardial cushion behind. Behind the posterior endocardial cushion a marked band of muscle extends from the wall of the sinus venosus to the interventricular foramen where it spreads over the inner walls of the two ventricles as shown in fig. 9. This is the sino- or atrio-ventricular bundle (bundle of His). These structures I shall describe in the heart of embryo No. 353 which is an unusually well preserved specimen, perfect in every respect and of the right stage of development for this purpose.

Embryo No. 353 is 11 mm. long with a well formed arm and hand plate. A profile outline of the embryo may be seen in the figure by Evans.¹³ The sections are 10μ thick in a coronal direction and slightly oblique, that is, they strike the heart transversely. The heart which has been modeled in wax is 1.7 mm. wide and 2.2 mm. long. The apex is cleft as is so often the case in this stage of development.¹⁴ The septum aorto pulmonale is complete and the anterior and posterior cushions are fully united into a single mass



Fig. 11 View from below of a model of the heart of an embryo 11 mm. long (No. 353). $\times 50$. The ventricles have been cut off. The connective tissue septa are colored yellow.

of connective tissue. This mass extends to a point up in the atrial septum on its dorsal side, to the left valve of the opening into the sinus venosus. The complete union of the two cushions has obliterated the foramen ovale I and the foramen ovale II is well above the common fibrous process of the united cushions. A view from below, that is after the apex of the heart is cut off,

¹³ Evans, Keibel-Mall Handbuch, Bd. 2, fig. 469.

¹⁴ Mall, Bifid apex in the human heart. *Anat. Rec.*, vol. 6, 1912.

shows that the cushions mark the borders of the medial sides of the right and left ostia, these being notched to indicate the extent of the anterior and posterior cushions. This is well shown in Fig. 11 which was drawn from the model. On the dorsal side, the posterior cushion extends well down the posterior border of the interventricular foramen, that is it makes part of the border of the septum of the ventricle. These are the primary connections of the two endocardial cushions. There are also secondary connections which in a measure involve the valves lateral to the ostia.

On the lateral side of either ostium there is a rounded endocardial thickening which marks the beginning of the lateral valves. These are already observed in Embryo 422, 9 mm. long (fig. 7). To anticipate the description I may state that the right cushion marks the center of the anlage of the anterior and posterior cusps of the tricuspid valve, and the left the anlage of the posterior cusp of the mitral valve as seen in fig. 11. The septum aorto pulmonale soon blends with the cushions through a dorso-lateral wing which is divided into two branches to encircle in part the right venous ostium, one of which blends with the right lateral endocardial thickening, and the other, the medial, blends with the anterior process of the medial valves now represented by the right lower wing of the anterior endocardial cushion. It is thus seen that through the blending of the septum aorto pulmonale with the right side of the anterior endocardial cushion, the right venous ostium is nearly encircled by endothelial connective tissue. This connection may still be seen in the adult heart where the septum aorto-pulmonale (the tendon of the conus) is found to blend with the fibrous ring of the right ostium at the anterior border of the attachment of the medial cusp of the tricuspid valve.

The large space marked by the interventricular foramen at the root of the aorta remains constant in all subsequent stages of development and is termed by Quain¹⁵ the vestibule of the aorta. This name, which is appropriate, I shall adopt and use in my description. The vestibule in fig. 11 is common to both ventricles,

¹⁵ Quain's Anatomy, 10th Edition, vol. 2, fig. 317.

but as the septum of the ventricles and the septum aorto pulmonale approach each other more and more to form the permanent membranous septum, the vestibule becomes transferred to the left ventricle as may be seen in figs. 16 and 17. An open inter-ventricular foramen in the adult always communicates between the aortic vestibule and the space below the medial cusp of the tricuspid valve, as is clear by observing Spalteholz's figure.¹⁶

The topography in the wall of the left ventricle is much easier to define. The common endocardial mass borders the left venous ostium and each of its two horns are continuous with pronounced muscular bands, the papillary muscles, which extend to the more solid muscular wall of the heart. In their course from the valve to the outer wall of the heart muscle the papillary muscles communicate continuously with the trabecular system. Both the anterior and posterior papillary muscles connect with the lateral valve which is being extended around the left ostium by an "undermining" process, already well described by His. So in this early stage of development the anterior papillary muscle unites the anterior tip of the medial and lateral valves (anterior and posterior B.N.A.) with the anterior wall of the left ventricle, and the posterior muscle unites the posterior tip with the posterior wall of the heart as seen in fig. 11. The vestibule of the aorta connects the aorta with the left ventricle; it is encircled by the border of the ventricular septum. In the course of time the border of the ventricular septum unites with the septum aorto pulmonale and thus finally separates the two ventricles of the heart. When viewed through the aorta the muscular interventricular septum usually makes in the adult the right border of the vestibule but often it projects into the vestibule as is normally the case in the pig and the ox. In such specimens, as well as in the pig and the ox, the right semilunar valve arises directly from the interventricular muscular septum. This shows to what extent the valves must 'sink into' the bulbus in passing from the stage represented in No. 353 to the adult form.¹⁷

¹⁶ Spalteholz, Hand atlas, vol. 2, fig. 420.

¹⁷ The normal position of the membranous septum is described in an article on aneurysms arising from it, in the *Anatomical Record*, vol. 6, 1912.

The anatomy of the heart of embryo No. 353 may serve as a basis to describe the final closure of the interventricular opening and the formation of the membranous septum. In this specimen the septum aorto pulmonale has grown down to the interventricular foramen and blends with the right tip of the anterior endocardial cushion which is lodged in the foramen. The aortic septum also extends to the lateral side of the right venous ostium and the posterior cushion extends down the anterior border of the ventricular septum. So the interventricular foramen is



Fig. 12 Coronal section. Embryo 13 mm. long (No. 175). $\times 40$.

bounded above by the union of the septum aorto pulmonale and the anterior cushion, in front by the septum aorto pulmonale, behind by the extended portion of the posterior cushion and below by the muscle of the ventricular septum.

In embryos 109 (10.5) and 317 (12.5) the ventricular septum is much more developed than in the stage just described. In these two specimens the fibrous tissue forming the septa and valves is much as in No. 353, but the muscle wall has grown up and nearly closes the interventricular foramen. However, there

is still a free communication between the right ostium, with the right ventricle and the vestibule of the aorta. The interventricular foramen is somewhat smaller in No. 175 (13 mm.) which is cut in a more fortunate plane to illustrate this point than the



Fig. 13 Sagittal section. Embryo 14 mm. long (No. 144). $\times 40$.

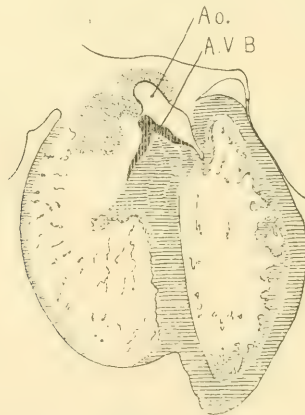


Fig. 14 Coronal section. Embryo 17.2 mm. long (No. 424). $\times 24$.

other two embryos (fig. 12). The opening is gradually becoming smaller in the following embryos in the order of their enumeration, No. 423 (15.2), 144 (14, fig. 13), 424 (17, fig. 14). In 390 (15.5) it is uncertain whether it is closed or not, in 409 (16) it is

just closed, and in 432 (18) it is closed but its connection with the right ventricle is still indicated. That the order of development does not correspond with the length of the embryo is due to the method of measuring; No. 144 was measured on the glass slide. But a comparison with the profile drawings of these specimens shows that the order of closure of the foramen corresponds with the degree of development of the external form. In No. 423 the interventricular foramen (0.1 mm. in diameter) is situated well anterior, at the point of junction between the septum aorto pulmonale and the right wing of the anterior endocardial cushion. It is under the medial cusp of the tricuspid valve in exactly the position taken by the atrio-ventricular bundle. In No. 424 the foramen is barely 0.02 mm. in diameter, and were not the vascular system injected with india ink the opening would probably be overlooked. It is present in but a single section. Here it is again located with the right limb of the atrio-ventricular bundle below the medial cusp of the tricuspid valve well anterior. On the left side it communicates with the vestibule of the aorta exactly in the position the atrio-ventricular bundle lies in the adult heart. That this is of significance will be considered when the atrio-ventricular bundle is discussed. In this stage the posterior cusp of the aortic valve still lies somewhat distant, but as the valves sink deeper and deeper into the vestibule of the aorta the position of the interventricular opening comes to lie behind the posterior cusp adjacent to the left limb of the atrio-ventricular bundle. In an adult heart with a patent interventricular foramen I have found the atrio-ventricular system streaming through this opening, thus showing that there is an association between them.

The atrio-ventricular valves are not as difficult to trace in their development in the successive stages as has been that of the formation of the membranous septum. In the youngest embryos, that is those under 3.5 mm. long, the endocardial connective tissue which was quite equally distributed in the earliest stages has gradually rearranged itself, first becoming less pronounced in the atria and then becoming well dove-tailed with the trabecular system in the ventricle and bulb. As soon as the atrial canal is well formed the endocardial connective tissue arranges itself

there in the form of two cushions the anterior and posterior endocardial cushions. In the bulb two ridges are also formed which ultimately give rise to the septum aorto pulmonale, but as this latter structure has been considered by Greil and by Tandler, I have taken it up only in so far as it bears upon the formation of the membranous septum.

In the embryo 3.9 mm. long (No. 463) the two endocardial cushions of the atrial canal are well formed (fig. 4). The posterior is short and reaches from the sinus to the ventricle, while the anterior is much more extensive for it reaches from the sinus also around the upper and anterior part of the atrium through the atrial canal to the bottom of the ventricle. In general they repeat that which is shown in Greil's fig. 3 taken from the heart of *Lacerta*.¹⁸ The two cushions are confined almost wholly to the left ventricle. However, the side of the lower tip of the anterior cushion passes through the interventricular foramen and continues as the anterior medial endocardial thickening of the bulb. A little later at 4.3 mm. (No. 148) the same arrangement is still seen except that the left lateral tip of each of the two cushions is more intimately attached to the trabecular system of the ventricle. These attachments mark the beginning of the anterior and posterior papillary muscles. The cushions gradually become more and more pronounced until the embryo is 7 mm. long (No. 2) when their lower right tips begin to enter the interventricular canal. With the formation of the septum primum the anterior cushion gradually approaches the posterior with which it ultimately blends (fig. 9). Before this takes place the septum primum is perforated forming the foramen ovale II; somewhat later the foramen ovale I is completely obliterated.¹⁹ By this time the two cushions have blended into a solid mass which obstructs the atrial canal, leaving on either side an ostium. The common cushion or valve mass is now wider than it is thick (fig. 15), hangs well into the left ventricle where its two corners or tips are well supported by the two papillary muscles (figs. 11 and 12). The right half rests upon the

¹⁸ Greil, Beiträge zur vergleich. Anat. u. Entwicklungsg. d. Herzens u. d. Truncus arteriosus d. Wirbelthiere. Morph. Jahrbuch, Bd. 31, 1903.

¹⁹ See also Greil, l.c., figs. 5 and 12.

inferior septum behind (the right posterior tip, projects well into the interventricular foramen, while the right anterior tip blends with the septum aorto pulmonale. It becomes clear, by comparing this stage with older ones, as well as with the adult (figs. 15 to 18), that this common central mass is destined to produce the medial valves of the two venous ostia, namely anterior cusp of the mitral valve and the medial cusp of the tricuspid valve.

But in addition to the anterior and posterior endocardial cushions there are also two lateral cushions well demonstrated by His and by Greil. One of these, the left lateral, is first seen in an embryo 9 mm. (No. 422) long, (fig. 7), but both are not well pronounced until the embryo is 11 mm. long (No. 353, figs. 11 and 16). At this time a lateral process from the septum aorto pulmonale reaches to and blends with the right lateral cushion. The left lateral is still sharply defined and isolated. The line of connection between the right lateral cushion and the septum is always marked in the adult by a tendon which passes into the aortic septum or by a small papillary muscle which holds the same position. This muscle is called medial papillary muscle by Henle.²⁰ Between the tendon or muscle and below the anterior tip of the medial cusp there is always a clear field, the bottom of which is formed by the membranous septum.

Semi-diagrammatic reconstructions of the tendinous masses at the base of the heart are shown in figs. 15 to 18. Fig. 15 is from the heart of embryo No. 163 (9 mm.) with the left lateral cushion from No. 422 (9 mm.) added. The muscular wall of the atrial canal is indicated and the connective tissue is stippled. Fig. 16 is from embryo No. 353 (11 mm.) which shows that the origin of the aorta has shifted well into the left ventricle. In fig. 17, No. 296 (17 mm.) the sinuses marking the three semi-lunar valves are indicated upon the two wings of the septum aorto pulmonale, and upon the anterior endocardial cushion. Fig. 18 shows the arrangement of the connective tissue at the base of the adult heart. The parts of the valves which are formed by

²⁰ Henle, *Gefasslehre*, II Auflage, 1876, fig. 33. This tendon is constant, in fact the most constant of all of the attachments of the valves in the right ventricle. It is pictured in all anatomies, but is not recognized in the B.N.A.

the undermining process are indicated by cross hatching. The course of the atrio-ventricular muscle bundle dividing within the interventricular foramen and crossing the ventricular septum,

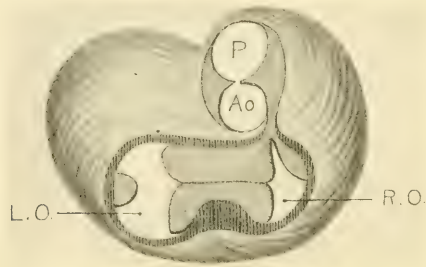


Fig. 15 Semidiagrammatic reconstruction of the heart of an embryo 9 mm. long (No. 163). The left lateral endocardial cushion has been added from another embryo of 9 mm. (No. 422).

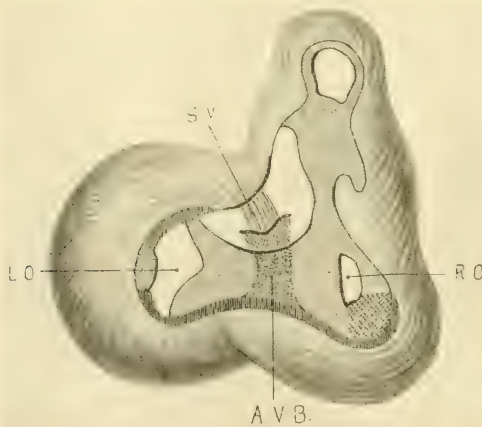


Fig. 16 Semidiagrammatic reconstruction of the heart of an embryo 11 mm. long (No. 353). The muscle encircling the atrial canal is reduced and the atrio-ventricular bundle is seen passing behind the endocardial cushions to spread over the ventricular septum below.

is also shown. These figures indicate clearly the fate of the primary dividing masses of connective tissue at the base of the heart in the embryo.

In young hearts the septum aorto pulmonale encircles the right ostium as shown in fig. 17 and from it the tricuspid valve arises. In the adult the septum aorto pulmonale blends with the anterior

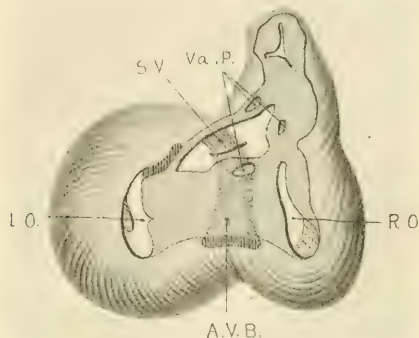


Fig. 17 The same as fig. 16 from the heart of an embryo 17 mm. long (No. 296). Most of the muscle wall of the atrial canal is absent. The atrio-ventricular bundle is shown and the semilunar valves of the aorta are indicated.

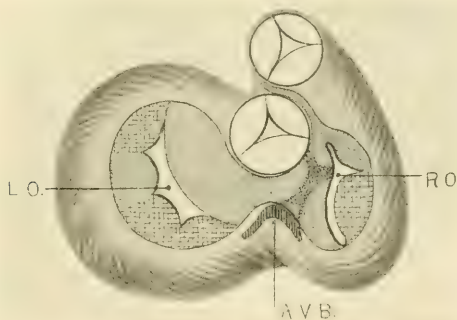


Fig. 18 Sketch of the base of an adult heart showing the valves. The atrio-ventricular bundle is shown. The figure may be compared with figs. 15, 16 and 17.

end of the medial and anterior cusp of the tricuspid valve, while the same tip of the valve is tied to the conus by the medial tendon which frequently enlarges into a papillary muscle. In following its development it is found that this tendon marks the primary

union of the septum aorto pulmonale to the valve and in its subsequent development is separated from the septum as the valve enlarges. The beginning of this process is shown in fig. 19 which is from a section of embryo 86 (30 mm.). It is here seen that the valve mass is enlarging and that its lower border is being separated from the muscle of the right ventricle as the tendinous cords are forming. In so doing the medial papillary muscle is forming and at the same time the trabeculae which encircle the base of the

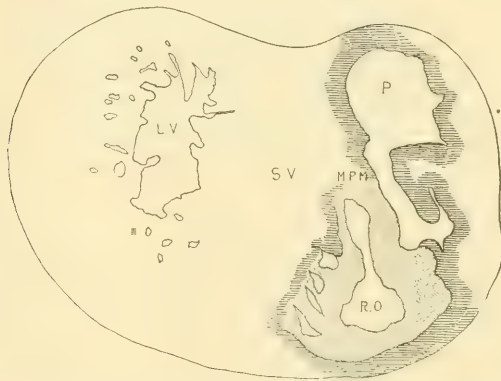


Fig. 19 Section through the conus and right ostium of the heart of an embryo 30 mm. long (No. 86). $\times 20$. The attachment of the tricuspid valve to the septum aorto pulmonale and the ventricular septum is shown.

right ventricle as shown in fig. 11, become fused to produce the crista supraventricularis.²¹ This at first ends in the septum aorto pulmonale but in the adult it passes around and over the medial tendon and passes down the anterior inner wall of the right ventricle. In the embryo the moderator band arises just below the medial cusp of the tricuspid valve, but in its further development is shifted towards the apex where it binds the large papillary mus-

²¹ The 'crista' is correctly figured in Toldt's Atlas, fig. 946, while in Spalteholz, fig. 424, it is pictured as extending down to the base of the large papillary muscle. It is this extension which contains the right limb of the atrio-ventricular bundle and therefore must represent the moderator band. A study of the development of this portion of the heart shows that this is the case.

cle with the extension of the crista supraventricularis.²² In the ox, pig and sheep, however, it retains its original position and contains the right limb of the atrio-ventricular bundle.

It is thus seen that the medial cusp of the tricuspid valve is attached in front by the medial tendon (or muscle), and behind by the large papillary muscle, and the inequality of these two structures accounts for the double appearance of the lateral valve. In reality no true tricuspid valve is present and correctly speaking there is no tricuspid valve. Both are bicuspid with medial and lateral cusps. Both are tied down by two muscles, the two papillary muscles on the left side and the large papillary muscle and the median tendon on the right side.

The atrio-ventricular cushions expand not only by their own growth but to a greater extent by a process of undermining the ventricle wall all around the venous ostia. To what extent this burrowing has taken place is marked by the attachment of the valves to the muscle walls of the heart. The tendons nearest the tips of the valves were the first to form, while those nearer the bases of the valves were formed subsequently. This has all been demonstrated by His. By this process of undermining the attachment of the base of the valve to the wall of the ventricle, the atrial portion is telescoped into the ostia. The muscle of the atrium

²² Toldt's 'crista' (l.c., fig. 946) ends in the medial papillary muscle (anterior), which according to its development is correct. Spalteholz (l.c., fig. 424) extends the crista past the anterior tendon down to the base of the large papillary muscle (posterior). This connecting band contains the right limb of the atrio-ventricular band and is pictured by Tawara on his plate 7. Retzer (J. H. H. Bull., vol. 20, 1909, and Anat. Rec., vol. 6, 1912) associates the moderator band with the crista, in fact he says that when absent it is represented by the crista. He recognizes fully the meaning of the anterior (medial) papillary muscle, but when it is recalled that the right limb of the atrio-ventricular band passes on the posterior side of this muscle and the crista on its anterior, the identity of these two structures is disproved. In fact in the embryo the crista reaches to the medial muscle and the moderator band is considerably below it. In the adult heart this band is pushed to the base of the large (posterior) papillary muscle as pictured in Spalteholz and it contains the right limb of the atrio-ventricular bundle as shown by Tawara; this I have been able to confirm. His, who introduces this term 'crista' (Beitrag zur Anat. d. Mensch. Herzens, Leipzig, 1886, S. 9) is not clear in his description of its attachment to the septum. According to its development it should end in the septum aorto pulmonale, that is, at the point of origin of the medial papillary muscle.

extends into the atrial part of the valves, which at first is continuous through the tendinous cords with the ventricular muscle. At first the atrio-ventricular muscle connection is through the main wall of the ventricle, but as this is resolved into the trabecular system with the growth of the valves and the formation of the papillary muscles the connection between atrium and ventricle is through the tendinous cords which are at first muscular. Later with the degeneration of the muscle in the cords the muscular connection between the atria and ventricles was believed to have been broken down completely. In lower vertebrates the muscular connection between the atria and ventricles through the trabecular system remained throughout life, and the significance of this has been fully demonstrated by Gaskell²³ and His, Jr.²⁴ In man, however, all of the cords are converted into connective tissue, but the muscle of the atria extends well into the valves or it may reach to its free border (Kürchner).²⁵ It has also been stated that in some rare instances the tendinous cords of the mitral valve remain muscular in the adult (Oehl).²⁶ But the muscular connection through the valves is first interrupted at the free thin edges and later the muscular fibers in the tendinous cords disappear. The break in the muscular connection at the free edges of the valves will be considered in discussing the atrio-ventricular bundle.

Returning to the description of embryo No. 353 (11 mm.), it is possible to determine with considerable precision the various adult connections of the valves. The most definite valve is the anterior cusp of the mitral which is formed by the union of the left lateral tips of the anterior and posterior endocardial cushions. Each tip is here bound to the trabecular system by well formed muscle strands, one of which passes to the anterior wall of the heart and the other to the posterior as well as to the septum. It is clear that these two strands, which appear much earlier than in

²³ Gaskell, On the innervation of the heart with special reference to the heart of the tortoise. *Jour. Phys.*, vol. 4, 1884.

²⁴ W. His, Jr., Die Thätigkeit des embryonalen Herzens. *Arbeiten aus der med. Klinik zu Leipzig*, F. C. W. Vogel, 1893.

²⁵ Kürchner, *Wagner's Handwörterbuch*, vol. 2, 1844.

²⁶ Oehl, *Henle's Anatomie, Gefässlehre*, Braunschweig, 1876.

this specimen and continue throughout development, are the anlagen of the anterior and posterior papillary muscles respectively. In addition a heavy strand of tissue encircles the lateral side of the left ostium and unites the apices of the two papillary muscles. In the middle of this, protruding into the left ostium, is seen the conspicuous left lateral cushion already noted in an embryo 9 mm. long (No. 422, fig. 7). This arrangement corresponds with what is found in the adult heart. Each papillary muscle not only attaches itself to the tips of the anterior cusp but also to the tips of the posterior cusp. (It is confusing to use the B.N.A. terms anterior and posterior in naming the cusps when the embryological terms should be medial and lateral.) Between the two papillary muscles there is often a third or lateral papillary muscle, or numerous small muscles or the two muscles may be widened to fill this area. At any rate the structures found around the left ostium in embryo 353 represent fully what is present in the adult. We have in all cases two papillary muscles, each of which communicates freely with the tips of the two cusps of the mitral valves. On the dorsal side and in front of the left ostium the muscle of the atrium and ventricle is continuous, as is shown in fig. 16.

As the heart grows larger more chordae tendineae are formed necessarily nearer to the base of the heart. It follows that the primary condition found in No. 353 represents only the tips of the valves and that their subsequent enlargement is due partly to stretching of the anlage and partly to the undermining of the wall of the ventricle.

The united anterior and posterior endocardial cushions were projected at first into the left ventricle but subsequently its left tip becomes lodged in the interventricular foramen (fig. 11). To this smaller portion the septum aorto pulmonale attaches itself anteriorly and the inferior muscular septum posteriorly. The left half hangs freely within the left ventricle throughout life, forming a loose flap or sail which is suspended between the left venous ostium and the vestibule of the aorta. While the semilunar valves of the aorta are somewhat distant from this flap in embryo No. 353, later on they are pushed down to it so that the aorta becomes finally attached to its base (fig. 17).

On the right side of the heart the septum aorto pulmonale soon blends with the lateral tip of the anterior cushion and by the time the septum is complete, as in No. 353, it divides, one portion of which is attached directly to the left anterior tip of the common cushion and the other encircles the right venous ostium and blends with the right lateral cushion. The posterior side of the ostium is formed by trabeculae which pass from the lateral cushion mainly to the muscular septum and others which course symmetrically into the trabecular system of the ventricle. It is by no means easy to determine three systems to correspond with the three cusps of this valve. Neither is it easy to recognize the three cusps in the adult unless we associate the medial papillary muscle, which is constant, with the anterior cusp and the large papillary muscle with the posterior cusp. If this is done it is possible to name the valves in the heart of embryo No. 356 as follows (figs. 15 to 18):

The median cusp is attached in this embryo to the septum aorto pulmonale in front and to the muscular septum behind. It has corresponding attachments in the adult. The anterior cusp is attached partly to the septum aorto pulmonale and to the large papillary muscle. The posterior cusp is attached to the large papillary muscle. If this division is correct the lateral cushion belongs to the anterior cusp while the posterior cusp is developed entirely from the lateral side of the ventricle. The medial papillary muscle is constant and always extends as a muscle, or as a tendon, from the region of the septum aorto pulmonale (tendon of the conus) to the anterior cusp which is marked by a similar strand of tissue from the septum to the lateral cushion in the embryo. That this is the case is shown in sections of older hearts, one of which is given in fig. 19. The posterior cusp, over the large papillary muscle, is of irregular formation and by a process of exclusion the muscle strands in the embryo which encircle the right ostium behind the right cushion, should give rise to it. At any rate the right and left ostia of numerous hearts of embryos less than 20 mm. long, are slit-like and similar, so that it is impossible to state that one is encircled by a tricuspid valve. Each is bordered by medial and lateral valves, and each medial and lateral

valve is bound to the ventricle by anterior and posterior papillary muscles. In the course of time the valves and papillary muscles will be renamed to correspond with their development, which will also accord with the adult condition.²⁷

C. THE ATRIO-VENTRICULAR BUNDLE

No complete description can be given to the formation of the tricuspid and mitral valves without considering the fate of the muscular wall of the atrial canal. This at once defines definitely the atrio-ventricular bundle which is embryologically the remnant of the auricular canal after the greater portion of its muscular wall has been destroyed by the formation of the valves.

His, Sr., described the breaking down of the muscular wall of the atrial canal in small embryos and states that it is nearly broken down in an embryo 13.8 (?) mm. long. Later His, Jr., showed the presence of a muscle bundle connecting atrium with ventricle in the new-born child and discusses the connections between atrium and ventricle in lower animals as well as in the embryo. He noted that the character of the heart beat changed in the chick at the time the atrial canal is forming, at the time the muscular connection between the atria and ventricles is reducing.²⁸ It is easy to read into this paper that His thought that the muscular connection between the atria and ventricles never does break down completely, although he never states it. Were there a complete separation of the muscle wall of the atria and ventricles before the formation of the atrio-ventricular bundle is formed heart block should occur in the embryo. That the atrio-ventricular bundle is a remnant of the primary connection in the embryo is mentioned by Tandler as a possibility but he believes it improbable. He is

²⁷ Direct dissection of the heart of an embryo 30 mm. long (No. 90, a) shows that the medial valves of the two ostia form a saddle hanging over the ventricular septum. Instead of single lateral cushions double lateral cushions are present on both sides, the posterior being larger than the anterior on the right side. A similar condition is sometimes seen in serial sections. In case the ventricles are cut through transversely the valve system can be beautifully seen from below, and this method of investigation will probably settle this question definitely.

²⁸ His, Jr., l.c., p. 19.

rather inclined to think that it is a new formation, as has been asserted by Retzer.²⁹

In following the atrio-ventricular bundle in the foetus Fahr³⁰ found it in one of 160 mm. long, Tawara³¹ in one 100 mm. long, Mönckeberg³² at 75 mm., Tandler in human embryos 28.5 and 19 mm. long, and Retzer in the pig from 15 to 20 mm. long. His, Sr., states that the muscle wall between the atria and ventricles is nearly broken down in an embryo 13.8 (?) mm. long. It is evident that if the bundle is a new formation it must appear in embryos about 15 mm. long. However, in order to anticipate my own report upon this subject it may be stated that the bundle is not a new formation but is the remnant of the wall of the atrial canal after its anterior and lateral sides have been broken in the formation of the tricuspid and mitral valves. The portion on the posterior wall which connects the sinus with the ventricle never breaks down; early in development it shows changes in structure which differentiate it from the rest of the heart muscle. It holds a constant position just behind the posterior endocardial cushion immediately over the septum of the ventricle, a position which is marked at first by the interventricular foramen and later by the membranous septum.

The atrial canal is first well formed in an embryo 3.9 mm. long (No. 463); it is now markedly constricted and is pretty well filled up by the two large endocardial cushions (fig. 4). Its outer wall is uniformly muscular throughout. There is practically no changes in subsequent stages until the lateral endocardial cushions make their appearance (fig. 15), both of which are present in an embryo 11 mm. long (No. 353, fig. 16). At this time there is a break in the region of the left cushion, and possibly over the right cushion, as well as in the bottom of the bulbo-ventricular groove, that is between the aorta and the left ventricle. At 13 mm. (No. 406) the break is more marked over the left endocar-

²⁹ Retzer, *Anat. Rec.*, vol. 2, 1908.

³⁰ Fahr, *Virchow's Archiv*, Bd. 188, 1907.

³¹ Tawara, *Das Reizleitungssystem des Säugethierherzens*, Jena, 1906.

³² Mönckeberg, *Untersuchungen über des Atrioventrikulärbündel in menschlichen Herzens*. Jena, 1908.

dial cushion, there are several breaks on the right lateral side, a large one just behind the aorta and one on the medial dorsal side of the left ostium. In other words the muscular connections between the atria and ventricles are as follows: A large one in front of and a large one behind the left ostium, a number of small ones lateral to the right ostium and a large one from the sinus to the ventricle dorsally just over its inferior septum. In another embryo of the same stage of development (No. 317, 12.5 mm.) there are two marked muscular connections, one in front of each of the ostia. There is a very marked one from the sinus to the inferior septum below. This is composed of muscle fibers which arise as two horns to which the dorsal muscle fibers of the atria stream. This is clearly the atrio-ventricular muscle, as shown in fig. 17. From now on it is the chief muscular connection but for some time additional connections are often seen. For instance in embryos 14 mm. (No. 144) and 15.2 mm. (No. 423) there is an additional connection just in front of the right ostium, in embryos 16 mm. (409), 18.5 mm. (431) and 20 mm. (368) there is an additional connection just in front of the left ostium. In a number of specimens the atrio-ventricular bundle is not confined to the region immediately over the inferior septum but is spread out over the dorsal side of the left ostium (432, 18 mm.; 431, 18.5 mm.; 368, 20 mm.), while in one specimen 21 mm. long (460) there is a single additional strip on the left lateral side. In general, however, the main bundle which is at first broad and associated more with the left ventricle than the right, gradually becomes constricted so that it is well formed and rounded by the time the embryo is 20 mm. long. It is beginning to be isolated at 11 mm. and well separated at 13 mm.

The position of the bundle is shown in sagittal sections in figs. 9 (No. 113, 8 mm. long), 13 (144, 14 mm.), 20 (390, 15.5 mm.), 21 (432, 18 mm.), 22 (431, 18.5 mm.) and 23 (368, 20 mm.). It is clear in all of these hearts that the muscle connection is from the sinus between the posterior cushion and the annulus fibrosus into the septum of the ventricle. This is its position in the adult heart. In transverse sections the bundle is shown in figs. 8 (No. 2, 7 mm.), 24 (353, 11 mm.) and 26 (317, 16 mm.). Here again it

is clear that the bundle lies just back of the endocardial cushion. Coronal sections of the heart giving the position of the bundle are shown in figs. 27 (46, 21 mm.), 29 (409, 16 mm.) and 30 (423, 15.2 mm.). In these figures it is clear that the bundle passes below the medial cusp of the tricuspid valve and between it and the interventricular septum. The extreme dorsal connection is shown in fig. 30. In earlier stages while the interventricular foramen is still present, it is easily seen that the bundle forms its wall as indicated in figs. 12 (175, 13 mm.) and 14 (424, 17.2 mm.).

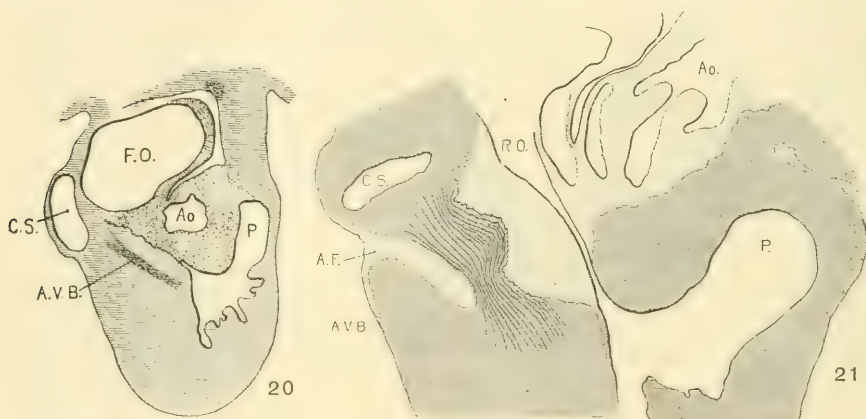


Fig. 20 Sagittal section. Embryo 15.5 mm. long (No. 390). $\times 18$.

Fig. 21 Sagittal section. Embryo 18 mm. (No. 432). $\times 40$.

These sections show definitely that the bundle is present in the youngest hearts after the atrial wall has begun to break down, and that its course is exactly the same as it is in the adult. After the embryo is over 20 mm. long the bundle is easily seen, as has been demonstrated by other observers. Figs. 15 to 18 gives a summary of what I have stated.

The additional strips above connecting atria with ventricles in various stages in embryos less than 20 mm. long may be of significance in view of Romberg's and Kent's observations. Romberg³³ found muscular connections between the anterior and pos-

³³ Krehl and Romberg, Ueber die Bedeutung des Herzmuskels, etc., Arbeiten aus der med. Klinik zu Leipzig, 1893, p. 72; and His, Jr., Die Thätigkeit des embryonalen Herzens, Ibid., p. 25.

terior cusps of the tricuspid valves. Kent³⁴ found such bundles in the left wall of the heart as well as in the right wall in young rats and young rabbits. It is possible that some of these strips

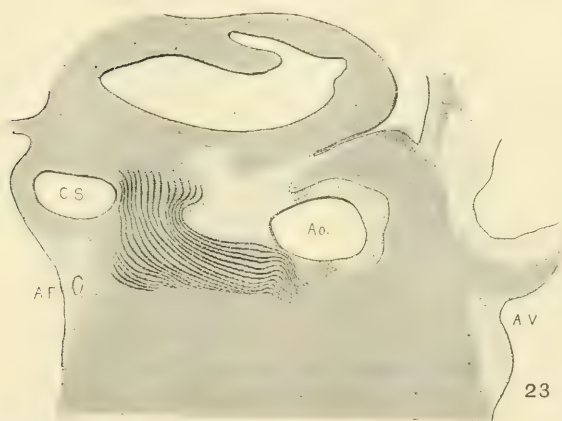


Fig. 22 Sagittal section. Embryo 18.5 mm. long (No. 431). $\times 40$.

Fig. 23 Sagittal section. Embryo 20 mm. long (No. 368). $\times 40$.

may be constant or they may be variations. At any rate their presence has not been established as has been the atrio-ventricular muscle of His.

³⁴ Kent, Researches on the development of the mammalian heart. *Journal of Physiology*, vol. 14, 1893.

So far I have shown that there is a band of tissue apparently muscular, which connects the sinus, or atria, with the ventricle. At first it is broad but it gradually becomes constricted. In an embryo 12.5 mm. long (317) the muscle fibers of the atria stream towards the connecting muscle as two horns, somewhat later it arises from a thickened mass in the neighborhood of the sinus and finally from the mass which has become converted into a nodule. About this time according to His, Jr., the nerves begin to invade this region. However, I have been able to trace the ganglion cells through the septum to the valves in but a single specimen 34 mm. long (No. 249) which shows that the nerve fibers really do enter the bundle on its atrial side. To what extent they penetrate the ventricular portion is still undecided.

It is not easy to determine with certainty the destruction of the lateral and anterior walls of the atrial canal, and my statements rest upon repeated studies of this region in all of my embryos. Not only does the endocardial thickening play a rôle in this process but the connective tissue of the epicardium also plays a part, as His, Sr., has shown. To what extent the outer connective tissue plays a part is well shown in the reconstruction of an embryo 11 mm. long (353). The model shows that it forms a collar encircling entirely the heart between the atria and ventricles and also extends into the anterior and posterior longitudinal sulci. On the two lateral sides the encircling connective tissue is drawn into the valves as they become larger and this process completes the separation of the atrial and ventricular musculature. Between the atria and the aorta it forms a large plug which soon comes into apposition and blends with the anterior endocardial cushion. Behind it encircles the atrio-ventricular bundle so that this bundle becomes lodged between the posterior cushion and the outer connective tissue. The relation here found corresponds with the position of the bundle in the adult, for it then lies behind the medial cusp of the tricuspid valve which arises from the endocardial cushion, and the right annulus fibrosus, which arises from the outer connective tissue.

That the bundle differs in structure from the rest of the muscle of the heart of the foetus has been shown by Möntekeberg, who

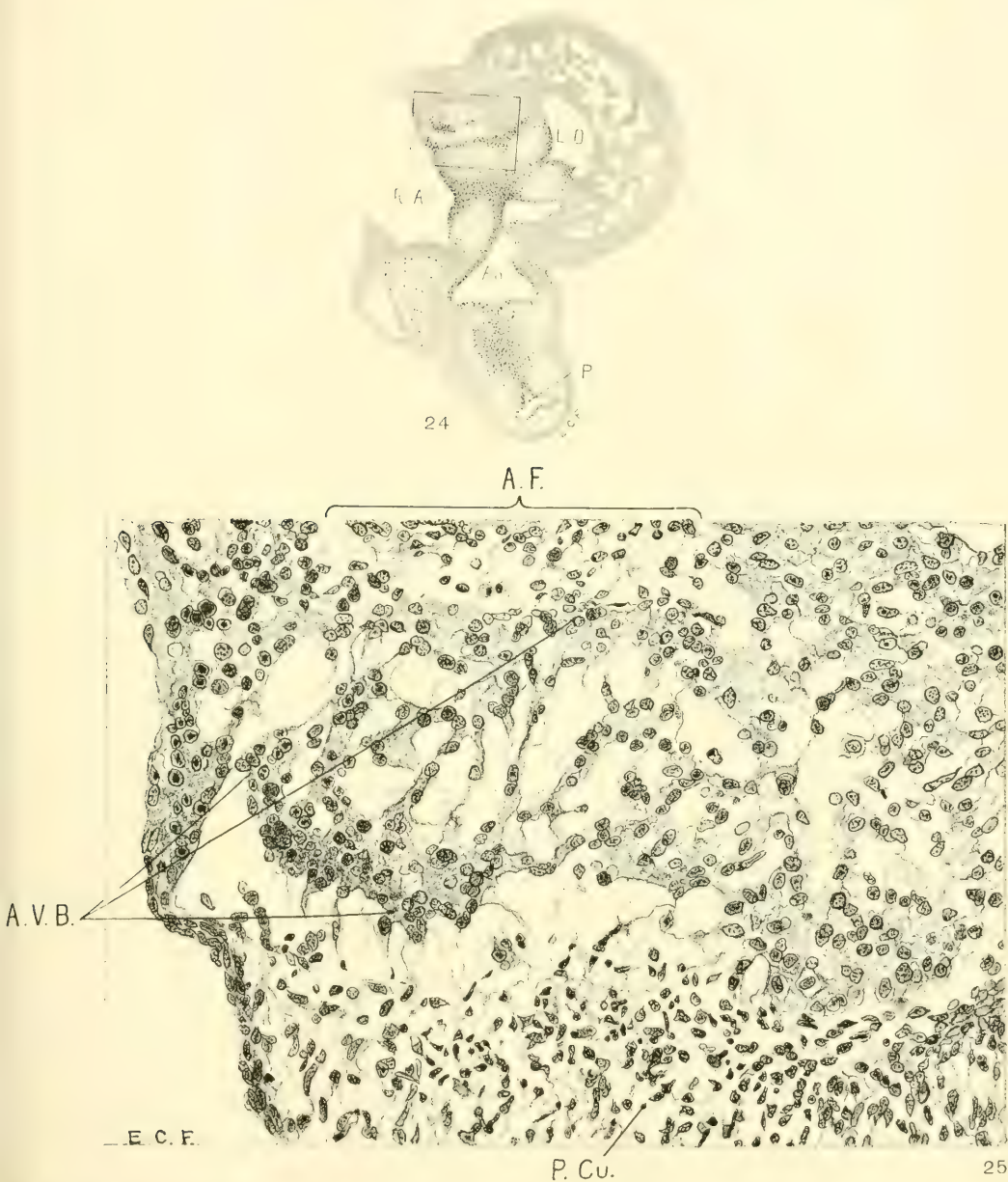


Fig. 24 Transverse section of the heart of an embryo 11 mm. long (No. 353). $\times 33$.

Fig. 25 Enlarged drawing of the square shown in Fig. 24 including the atrio-ventricular muscle. $\times 360$.

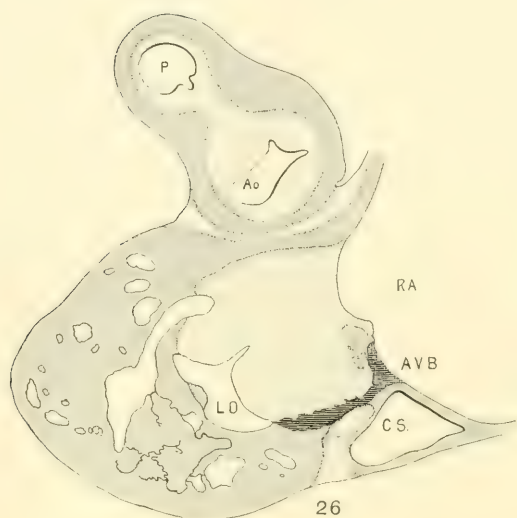
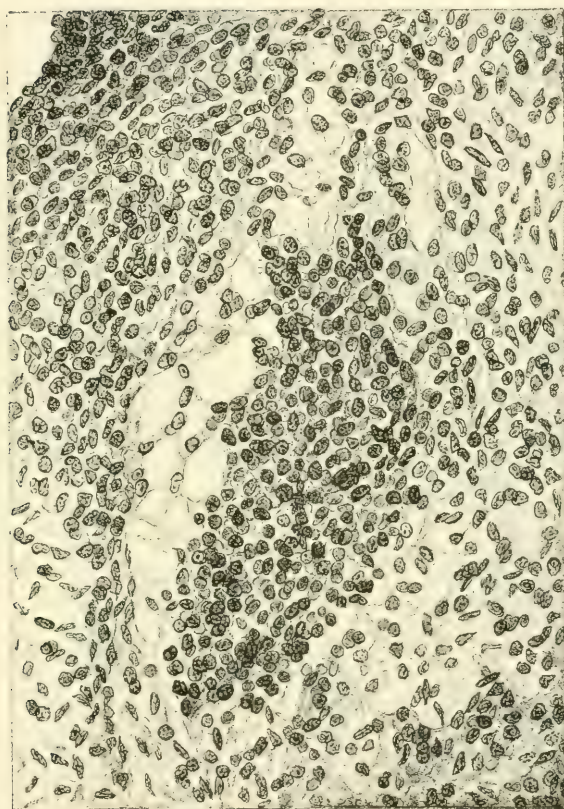


Fig. 26 Transverse section. Embryo 16 mm. (No. 317). $\times 40$.



Fig. 27 Coronal section through the heart of an embryo 21 mm. long (No. 460). A square is around the atrio-ventricular muscle.



A. E. H. 28

Fig. 28 Enlarged drawing of the atrio-ventricular muscle shown in Fig. 27.
× 360.

describes and pictures it in a specimen of the fifth month. Fahr states that the bundle is less pronounced in the foetus than in the adult but that its position is the same. Tandler found it in an embryo 20 mm. long as "distinguishable even under low powers by its staining properties." The nuclei are dark and the cell border stains faintly with eosin. At 20.5 mm. the cells of the bundle have become larger both in the atrium and the ventricle.

In suitable sagittal sections of the heart the atrio-ventricular bundle may be recognized in an embryo 8 mm. long (fig. 9) as a strand of tissue extending from the region of the sinus to the ventricle. This tissue is somewhat separated from the rest by delicate spaces and its cells stain somewhat more intensely than the rest. In embryo pigs 7 mm. long the bundle appears more like epithelium with a tendency towards vesicle formation in the immediate region of the sinus. In larger foetuses of the sheep the epithelial nature is most pronounced. In an embryo 11 mm. long (figs. 24 and 25) the numerous small spaces around the bundle encircle it from the region of the sinus just behind the posterior endocardial cushion, along its course behind the medial cusp of the tricuspid valve to the upper border of the ventricular septum. This same arrangement is found in another embryo (12.5 mm. long, fig. 6) cut in the same plane and stained in the same way. In this specimen the muscle fibrils are not present in the bundle but they are found in the muscle cells of the atria and the ventricles. In a sagittal section of an embryo 14 mm. long (fig. 13) the bundle is recognizable throughout its extent by its surrounding spaces and not by its staining properties. In 175 (fig. 12) the bundle can be followed into the two ventricles. The same structure and arrangement is made out in embryos 15.2 mm. (423), 15.5 mm. (390), 16 mm. (409, fig. 29) and 17.2 mm. (424, fig. 14). In older specimens the interventricular foramen is just closed and the bundle reaches to it from the region of the sinus. Two limbs are easily outlined one of which reaches to the moderator band of the right ventricle, which is pronounced in the human embryo.

The atrio-ventricular muscle is well lodged between the valve and the annulus fibrosus in an embryo 18 mm. long (432, fig. 21); it again extends into the moderator band. The same is observed

in another specimen of the same size and prepared in the same way (No. 43, 18.5 mm. long). At this stage the nerves have reached the region of the sinus as is seen in specimen (460, 21 mm. long). In this specimen the bundle can be followed by the spaces around it, figs. 27 and 28. Another specimen (368, 20 mm.), which is unusually well preserved to show the fibrillae of the heart muscle, shows that the degree of development of the atrio-ventricular bundle is the same as that of the rest of the musculature of the heart.

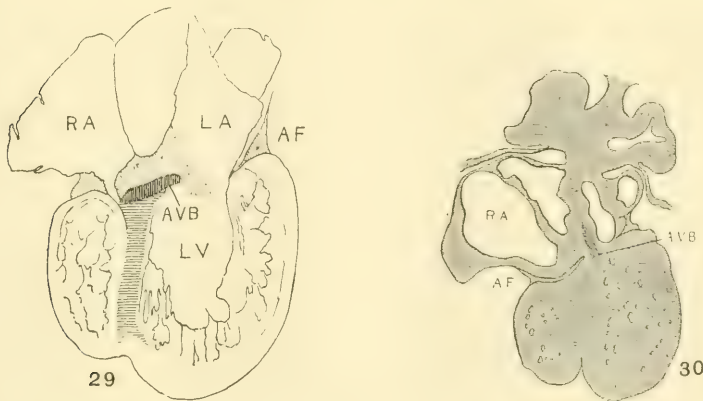


Fig. 29 Coronal section to show the position of the atrio-ventricular muscle. Embryo 16 mm. long (No. 409). $\times 18$.

Fig. 30 Coronal section, well dorsalwards, to show the extension of the atrio-ventricular muscle to the coronary sinus. Embryo 15.2 mm. long (No. 423). $\times 15$.

The spaces around the bundle in embryos from 10 to 20 mm. long appear to continue in the adult; possibly they form the bursa of the bundle described by Curran³⁵ and the spaces which encircle the Purkinje fibers. Brilliant injections of these have been made by Lhamon.³⁶

In an embryo 34 mm. long, No. 249, the atrio-ventricular bundle is chiefly muscular as it passes to the ventricle. Near the sinus

³⁵ Curran, A constant bursa in relation with the bundle of His. *Anat. Rec.*, vol. 3, 1909.

³⁶ Lhamon, The sheath of the sino-ventricular bundle. *Amer. Jour. Anat.*, vol. 13, 1912.

it is invaded by nerve cells. From now on there is a change in the structure which is well shown in two foetuses 50 mm. long (Nos. 84 and 96). In both the cells are more 'epithelial' in appearance and in the former this differentiated band can be followed into both of its divisions, one of which reaches to the moderator band. The limb of the bundle which passes to the left ventricle is most sharply defined. In a foetus 80 mm. long (No. 172) this bundle is easily made out and appears much as it does in other foetuses, or as it is in the adult.

It is thus seen that the atrio-ventricular bundle is present in an embryo 8 mm. long and that as soon as the muscle of the rest of the atrial canal is broken down it becomes outlined by encircling spaces which are now formed. Later it is composed of muscle which resembles much the rest of the heart musculature. When the nerves invade the septum of the atria the atrio-ventricular muscle shows marked histological changes which remind one much of the Purkinje fibers. These I have followed into both ventricles to the moderator band on the right side in the human heart, and through it in the pig. In the foetal sheep up to 15 cm. long much the same conditions prevail and by using a great variety of stains I was able to follow the atrio-ventricular bundle beyond the moderator band; no Purkinje fibers were found. As we now know the continuity of the bundle with the Purkinje system it is not remarkable that in early stages the structure of the main bundle simulates that of this system. In the adult heart Purkinje fibers are found throughout the ventricular portion of the atrio-ventricular bundle and this is all in harmony with what I have found. I can not leave the subject without expressing the suspicion that the differentiation of the Purkinje system is in some way due to the influence of nerves when they appear in the wall of the sinus.

D. MUSCULATURE OF THE LEFT VENTRICLE

It is practically impossible to unravel the musculature of ventricles from serial sections alone. However, it is possible to gain quite a clear picture of the muscle bundles by direct observation of the whole heart with the aid of the dissecting microscope. About two dozen hearts were removed from human embryos

ranging from 10 to 40 mm. in length and these were first carefully studied with various enlarging lenses including the binocular. It was found that the hearts from embryos over 15 mm. long could be dissected under the binocular to great advantage, especially after they had been stained in alum cochineal. So all of the hearts were stained and preserved in alcohol. No dissection was made hastily and during the preparation numerous sketches made. In the course of time after many attempts had been made I finally satisfied myself regarding the various stages of the development of the chief muscle bundles of the left ventricle.

The following specimens were dissected; the smallest embryo was an unusually good one from a tubal pregnancy. The heart

Number and length of embryos whose hearts were removed entire, studied and dissected

NO.	LENGTH	NO.	LENGTH
426	10	293	19
	12	263	23
360	14		25
434	15	118	25
90c	17	Davis	27
	17	33	27
90d	18	90a	30
90b	18	227	40
283b	19		

was removed and examined many times in various fluids by transmitted and reflected light. The best drawings were obtained from the stained heart while it was in 60 per cent alcohol. These are shown as diagrams in figs. 31, 32 and 33. It is noticed at once that most of the striations on the surface of the heart are circular, which is probably the arrangement of all the fibers in younger specimens. However, at the apex certain changes have taken place which fully account for the arrangement of the muscle bundles in the adult heart. In front of the heart, at the bulbo-ventricular furrow, the fibers leave the bulb and penetrate the medial side of the left ventricle, as indicated in fig. 31. Behind the fibers leave the surface of the left ventricle and enter the

inferior septum which is just beginning to form from below and behind (fig. 33). Thus it is seen that the vortex of the apex is laid down in the heart of an embryo 10 mm. long (fig. 32).

In a set of perfect serial sections of the heart of an embryo

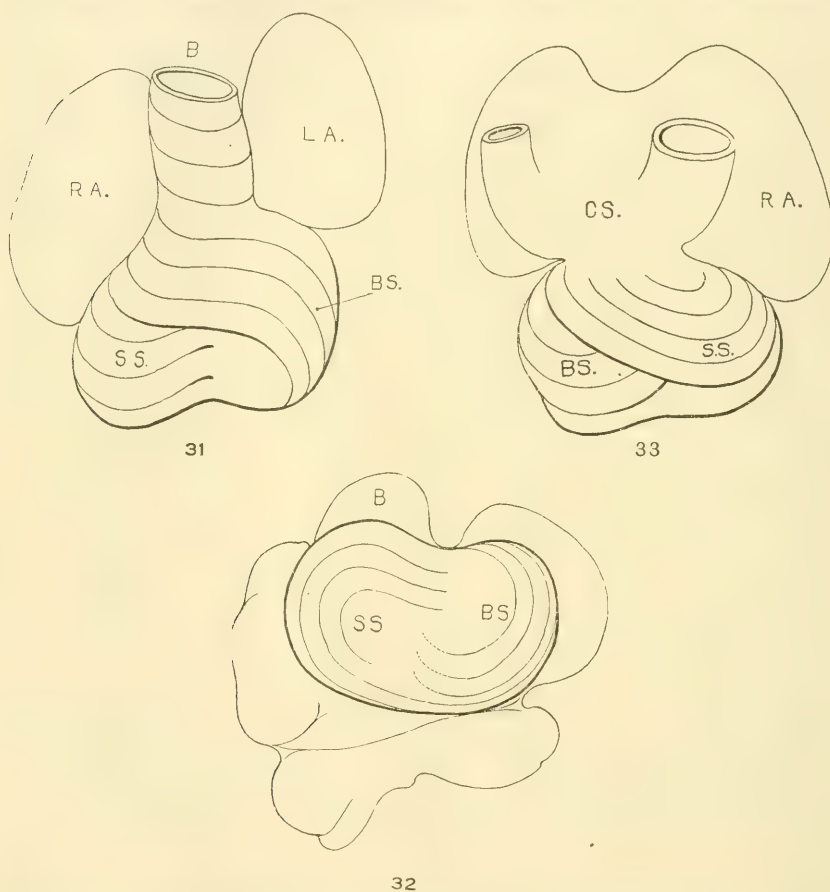


Fig. 31 Outline drawing of the front of the heart of an embryo 10 mm. long (No. 426). The course of the fibers is indicated.

Fig. 32 The same heart, viewed from below.

Fig. 33 The same heart, viewed from behind.

11 mm. long (353) no such interlacing of the fibers can be seen, excepting well up near the base of the heart where the fibers from the right side cross over the anterior longitudinal furrow, while

in the posterior longitudinal furrow the fibers from the left side enter the septum. There is every indication from the study of older specimens that the 'vortex' formation is at first well up in the middle of the heart and that only later it is pushed down to the apex of the left ventricle.³⁷ I was unable to determine this point with certainty from my sections. However, it is clear that in an embryo 3.9 mm. (463) long (fig. 1) the posterior bundle, that is the bulbo-spiral, enters the septum and forms its upper border just below the inter-ventricular foramen. Below this the sino-spiral should pass into the left ventricle in front, but sections do not show that the cells in this region of the heart are passing in any special direction. However, it is seen that the circular muscle of the left ventricle appears at the base of the heart and not at its apex. In this embryo the interventricular foramen is 0.15 mm. in diameter; in one 8 mm. long (113) it is 0.3 mm. in diameter and in one 11 mm. long (353) it is 0.4 mm. in diameter. This indicates that the ventricles grow longer by a down growth of their walls and not as an upgrowth of the inferior septum.³⁸ With this in view it is clear that the bulbo-spiral should be located near the base of the heart at the time of its earliest appearance.

In an embryo 12 mm. long the superficial fibers on the dorsal side of the heart, that is the bulbo-spiral, could be followed into the ventricular septum and the sino-spiral around the heart into the left ventricle. The structures showing the direction of the muscle fibers could only be seen after the whole heart had been stained with cochineal and methylene blue. In a specimen 14 mm. long (360) the heart was dissected from behind under the binocular microscope and the sino-spiral bundle was followed. These fibers passed on both sides of the ventricular septum in front to the septum aorto pulmonale, that is they formed the longitudinal bundles of the right ventricle; the spreading out of the sino-spiral in the left ventricle as well as the interpapillary muscle

³⁷ Mall, Bifid apex in the human heart. *Anat. Rec.*, vol 6, 1912.

³⁸ Flack (Further advances in physiology, New York, 1909) says that the ventricular septum grows downward as the ventricles expand and unite. The atrio-ventricular bundle is thus associated from the first with the oldest part of the septum, that is, its upper border which encircles the interventricular foramen.

bundles were also seen. A large band of fibers crossing the right ventricle was incorporated with this system; no doubt it is the moderator band, which is very pronounced in the embryo.

The two main bundles could be followed with precision in the heart of an embryo 17 mm. long, the bulbo-spiral passing into the upper part of the septum and the sino-spiral to the anterior wall of the left ventricle.

By the time the embryo reaches 25 mm. in length it is easy to demonstrate that the strands of muscle which were observed in younger specimens are destined to become the main muscle bundles of the heart in the adult. First of all there are fibers on the dorsal side of the heart which cross diagonally the posterior longitudinal sulcus to reach the apex of the right ventricle. From here they pass to the left ventricle and pierce it near the apex anteriorly to form the anterior horn of the vortex.³⁹ From this horn fibers pass on the right side of the ventricular septum to the septum aorto pulmonale, that is they are the longitudinal fibers of the right ventricle. Under the sino-spiral band loops of fibers are seen which encircle the left ventricle and end in the posterior triangular field. All of these loops enter the septum from behind and form the bulbo-spiral fibers. This arrangement was clearly demonstrated in a heart from an embryo 17 mm. long, as well as in specimens 25 and 27 mm. long (figs. 34 to 37). In one of these (No. 33) the sino-spiral turns upward at the apex of the right ventricle along the lower part of the anterior longitudinal sulcus and then enters the left ventricle. At this point the fibers blend with the trabeculae of the right ventricle and then give rise to the longitudinal fibers of the septum of the right ventricle. The bulbo-spiral bundles form loops reaching towards the apex of the left ventricle, that is, they are only an extension of the posterior triangular field. This, as has been shown in the previous study, remains in the adult as the circular fiber of the left venous ostium. At the extreme posterior border of the left ventricle there is a marked raphé to correspond with the base of the posterior papillary muscle much as in the adult pig's heart. The sino-spiral

³⁹ Mall, Amer. Jour. Anat., vol. 11, 1911.

loops now form the anterior horn of the vortex and the lower end of the loops form the posterior horn.⁴⁰

To sum up: The simple heart tube is made up of circular fibers which in a general way remain circular throughout development. After the heart is well kinked upon itself the fiber bundles around

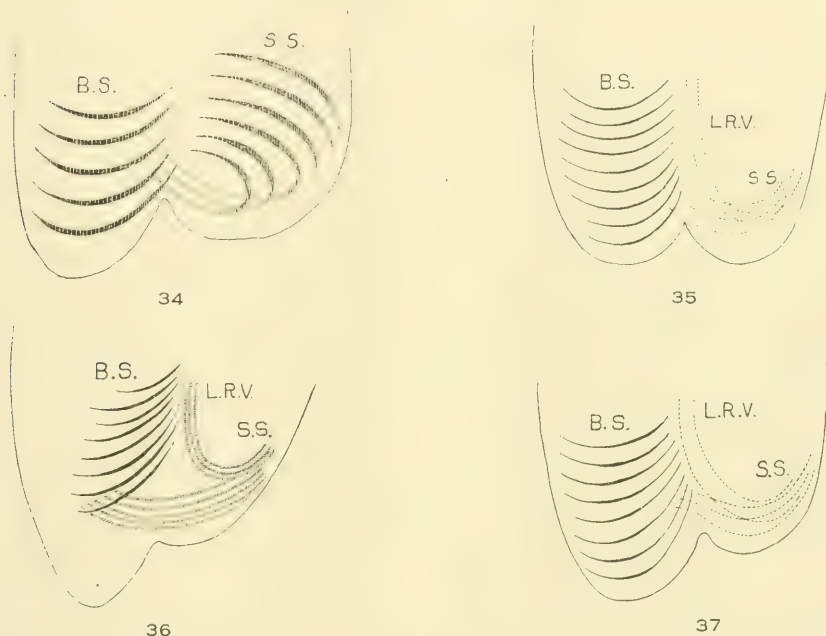


Fig. 34 Course of the fibers in the heart of an embryo 17 mm. long. The view is from behind.

Fig. 35 The same as fig. 34 from the heart of an embryo 25 mm. long. The fibers which pass to the base on the right ventricle are present.

Fig. 36 The same as fig. 35, from an embryo 27 mm. long.

Fig. 37 The same as fig. 35, from another embryo 27 mm. long (No. 33).

the venous and arterial ends remain circular but there are marked deviations at the apex which correspond with the kinking at this point. Those fibers that arise from the bulbo-ventricular groove in front encircle the left ventricle and enter the ventricular sep-

⁴⁰ His, *Anat., mensch. Embryonen*, Bd. 3, S. 177, makes a statement which is just the opposite of mine.

tum behind quite high up so that they form its border just below the interventricular foramen. Those that arise behind encircle the right ventricle and enter the heart in front of through the anterior longitudinal sulcus. Now as the heart grows by an extension of the ventricles downward these two encircling bands also grow and ultimately become interlocked at the apex of the left ventricle, the fibers from the bulb forming the bulbo-spiral band and the posterior horn of the vortex; the fibers from the sinus side, the sino-spiral band form the anterior horn of the vortex. As the sino-spiral band becomes larger and larger it is shifted over the greater part of the bulbo-spiral band on the posterior side of the heart. On the anterior side of the heart with the growth of the anterior longitudinal sulcus, the sino-spiral band gradually extends sending bundles to both sides of the septum, that is it constantly straddles the septum from its lower and under side. By this extension it binds together the inner walls, especially the papillary muscles, of the two ventricles with each other. These are the two strands shown in figs. 35 to 37.

The bulbo-spiral band first extends from the bulb over the left ventricle, and with its growth penetrates its wall, ultimately including its apex to end in the posterior horn of the vortex (figs. 31 to 33). In so doing it produces loops which constantly turn upon themselves to return to the base so that when viewed together the bulbo-spiral band appears as a series of telescoped loops of sheet fibers as is now well known. At the base of the left ventricle the fibers are more circular forming a thick fleshy mass on its dorsal side, the triangular field, which in the adult still marks the embryonic conditions; all loops of the bulbo-spiral band are but an extension of this field as is shown by the development of the fibers as well as by the architecture of this muscle in the adult.

THE DEVELOPMENT OF THE HUMAN PROSTATE GLAND WITH REFERENCE TO THE DEVELOPMENT OF OTHER STRUCTURES AT THE NECK OF THE URINARY BLADDER

OSWALD S. LOWSLEY

From the Anatomical Laboratory, Johns Hopkins University

ELEVEN FIGURES (THREE COLOR PLATES)

A review of the literature on the embryology of the prostate gland and other structures at the neck of the human bladder discloses a great diversity of findings. Much has been written about the middle lobe of the prostate and there seem to be two views very firmly held with regard to its development, one best expressed by Griffiths and utilized by Tandler and Zuckerkandl¹ to the effect that the middle lobe is an independent structure which may sometimes be lacking; the other supported by Pallin, Jores and others who believe that the middle lobe is always formed by ingrowths from the lateral lobes.

Griffiths² concludes from his studies: (1) That the middle lobe may be either present or absent at the time of puberty and in adult life before enlargement takes place. (2) That this lobe is independent, having glands of its own which open on parts of the hinder wall of the prostatic urethra. (3) That this region develops separately from the part of the urethra just mentioned in the same way as the lateral lobes do from the part of the urethra on each side of the verum montanum, and it is not the result of an extension back of gland tissue from the lateral lobes into the interval between the vasa deferentia beneath the neck of the bladder.

¹ Tandler and Zuckerhandl, *Folia Urologica*, Bd. 5, 1911, p. 587, in their discussion of prostatic hypertrophy state that the middle lobe is anatomically constant and believe that it is morphologically and embryologically independent.

² Griffiths, *Jour. of Anatomy and Physiology*, vol. 23, p. 374, 1889.

Griffiths³ states in another paper that there can be no enlargement of the third lobe unless there were gland tubules originally there. Any one of the lobes may enlarge without involvement of the others. This author states that enlargement does not take place in that part of the prostate behind the urethra and anterior to the verum montanum (posterior lobe).

Mansell Moullin⁴ states that the prostate is not an urinary organ but that the point of origin of the prostatic glands has simply been displaced in the course of racial development from the lining of the Wolffian ducts to that structure into which they open. The question of the median lobe, according to this author, depends upon the extent to which this displacement has occurred in each individual. So long as the glands are restricted to the prostatic sinus there is no median portion. In some instances a greater or smaller number are displaced towards the bladder and they not infrequently occupy the middle line. Usually they remain on the posterior wall of the urethra and form a more or less conspicuous median lobe. Exceptionally they make their appearance upon the anterior wall as well.

Keibel⁵ does not agree with this opinion and considers the prostate to be an urinary organ because its glands arise from the urethra above the openings of the Wolffian and Müllerian ducts.

Evatt⁶ in a study of a 12 cm. (crown-heel measurement) foetus which he considered to be three and one-half months of age, by means of a wax model described the middle lobe to be made up of branches of the two largest prostatic ducts which come together in the midline behind and these, with two smaller ducts immediately above them, form a centrally placed lobe above the level of the point of entrance of the genital cord. This he considers to be the middle lobe and consists of ducts derived from both sides of the prostate and cannot therefore be regarded as an azygos structure.

³ Griffiths, *Jour. of Anatomy and Physiology*, London, vol. 24, p. 236, 1890.

⁴ Mansell Moullin, *Jour. of Anatomy and Physiology*, vol. 29, 1895.

⁵ Keibel, *Archiv für Anatomie und Physiologie*, 1896.

⁶ Evatt, *Jour. of Anatomy and Physiology*, vol. 43, p. 314, 1908.

Gustaf Pallin⁷ summarizes the results of his study of human embryos aged three and four months by means of wax models as follows:

The prostate gland is deposited in male embryos in the third month by separation of the solid longitudinal folds on the outer side of the epithelial wall of the urethra. Three groups of prostatic tissue are distinguished, (1) Cranialwards from the genital cords lying dorsally, (2) Caudalwards from the genital cords lying dorsally, (3) Ventral.

Both of the first groups go out from the prostatic furrows. From the cranial the main mass of the base of the prostate will be composed; the third lobe seems not to be composed of independent glands but ramifications of the cranial glands can grow into the midline and then these become gland parts. The caudal dorsal structure forms the lateral and hind part of the side lobes. The ventral group at first occupies the greater part of the forward urethral wall. The number of its glands becomes reduced in the fourth month and it appears then in the midline as a forward lobe. In certain cases the reduction of this lobe amounts to complete atrophy.

This article is very extensively quoted and accompanied as it is by drawings from wax models and very accurate descriptions has influenced many workers.

Jores⁸ states that the middle lobe can be considered only as a glandular commissure connecting the two lateral lobes and not as an independent structure.

The posterior lobe is generally referred to in the literature as originating from ingrowths from the lateral lobes.

The number of tubules of the various parts of the prostate emptying their secretions into the prostatic urethra is usually stated in text-books to be between twenty and thirty, while the proportion of glandular tissue compared to the interglandular stroma is quoted by different authors to be from one-third or one-half (Kasuyoshi Nakasima) to five-sixths (Walker). The later writers seem to be agreed that the prostate begins to develop at about the third month of intra-uterine life. Kölliker thought that it was not present until the fourth month and Mihalkovics placed the fifth month as its beginning. The smooth muscle of the gland, according to the latter and also Tourneux, began to

⁷ Gustaf Pallin, *Archiv für Anatomie und Physiologie*, 1901.

⁸ Jores, *Virchow's Archiv für pathologische Anatomie*, Bd. 135, 1894, p. 224.

develop at the middle of the fifth month. Pallin, on the other hand, found the musculature to be developing at the fourth month.

Albarran⁹ describes a group of glands under the neck of the bladder which open into the urethra and whose tubules lie between the mucosa and the musculature. He calls this the subcervical glandular group and states that it varies greatly in different individuals and may be entirely lacking in some.

The bladder, trigonum vesicae, and internal sphincter have received a great deal of attention by writers on the anatomy of this region.

J. Griffiths¹⁰ believed the trigonum vesicae to be composed only of the innermost bands of muscular bundles of the bladder wall, while the outermost longitudinal bundles pass on to the neck of the bladder.

W. Waldeyer¹¹ called attention to the following facts in regard to the trigonum vesicae: (1) There is a separate development of its musculature which is continuous with the musculature of the ureters and the prostatic urethra. (2) There is an absence of a submucosa over the trigone. (3) It has a smooth, firm, thick layered mucous membrane.

Versari¹² concludes from his studies that normally the musculature of the trigonum vesicae is made up of (a) the trigonal portion of the internal sphincter, (b) part of the muscular layer of the ureters, and (c) the muscle bundles of their sheaths. In adults there are present in the trigonal region bundles which come from the muscular layer of the bladder.

Walker¹³ agrees with the above findings in part. He observes that from the ureter on each side a thick band of muscle passes down towards the urethra. These bands converge and unite so that this longitudinal muscle flows over the margin of the urethral opening in a continuous sheet. In the center of the triangle

⁹ Albarran, *Maladies de la prostate*, p. 526, 1902.

¹⁰ J. Griffiths, *Jour. of Anatomy and Physiology*, 1891, p. 535.

¹¹ W. Waldeyer, *Das Trigonum Vesicae*, *Sitzungsberichte der akademie der Wissenschaften in Berlin*, 1897, p. 732.

¹² Versari, *Ric. d. Lab. d. Roma*, vol. 13, 1907.

¹³ Walker, *Jour. of Anatomy and Physiology*, vol. 40, p. 190, 1906.

formed by these bands of muscle the fibers appear to interlace indiscriminately.

Delbet¹⁴ declares the trigonum vesicae to be an appendage of the urethral walls. Congenital absence of a ureter shows the trigonum to be lacking on that side. Passavant has described a case in which the trigone was entirely separate from the bladder wall.

In regard to the internal sphincter of the human bladder Versari¹⁵ concludes from his investigations that (1) The smooth muscle sphincter of the urinary bladder of man constitutes a structure by itself, which develops independently of the middle (circular) layer of the bladder, the circular muscle layer of the urethra and the musculature of the ureters. (2) The sphincter is made up of an urethral and a trigonal portion, and it is the urethral portion only which assumes the form of a ring surrounding the initial part of the urethra. The first groups of the fibers of the sphincter arranged in bundles correspond to the anterior arch of the urethral portion; from there immediately follow those of the urethral portion of the posterior arch, and these last are apparently those of the trigonal portion. The posterior arch of muscle extends little by little, with new bundles either upwards to occupy part of the trigonal area or downwards along the posterior wall of the urethra, so that it comes to have an extent much greater than the anterior. On the other hand, the older view held by Krause, Hyrtl, Gegenbauer and others is that the sphincter is a continuation downward of the circular musculature of the bladder.

The seminal vesicles begin to develop at about the third month (McMurrich).¹⁶ Pallin found that the ejaculatory ducts show no suggestion of smooth muscle at the sixth month. The colliculus seminalis of man is, according to D. Berry Hart,¹⁷ the analogue of the hymen and lower one-third of the vagina, the Müllerian ducts not being represented in the adult human male except by the hyda-

¹⁴ Poirier and Charpy: *Traite d'anatomie humaine*, vol. 5, p. 110.

¹⁵ Versari, *Ric. d. Lab. d. Roma*, vol. 13, 1907.

¹⁶ McMurrich, *The development of the human body*, Philadelphia, 1902.

¹⁷ Hart, *A contribution to the morphology of the human urino-genital tract*, *Jour. of Anatomy and Physiology*, 1901, p. 330.

tid of Morgagni and some rudiments near the testes. Primrose¹⁸ believes that the uterus masculinus must be looked upon as the homologue of the series of structures formed in the female by the fused portions of the Müllerian ducts. Minot¹⁹ states that in the male the Müllerian ducts remain rudimentary and their middle portions usually abort leaving the upper fimbriated ends to develop into so-called hydatids of Morgagni, and the lower or caudal ends to unite within the genital cord to form the so-called uterus masculinus, a rudimentary representation of the female uterus and vagina.

The prostates used in this investigation were obtained from Dr. Mall's collection of human fetuses which were preserved in alcohol or in formalin (4 per cent). They were imbedded in paraffine and cut, in series, being stained with haematoxylin and eosin or Mallory's stain. The measurements taken are crown-rump, and the ages of embryos are estimated according to the table in Keibel-Mall's *Manual of Embryology*.²⁰

Before taking up the discussion of the various specimens it seems best to state the terminology to be used. The various parts of the prostate gland will be referred to as follows: (1) The middle lobe, or that part of the gland which is situated between the bladder and the ejaculatory ducts under the floor of the urethra (prespermatic and post urethral). (2) Lateral lobes, or those parts of the gland which arise from the prostatic furrows and the lateral walls of the urethra and extend laterally and posteriorly from that structure. (3) Posterior lobe, or that part of the prostate which lies dorsal to the ejaculatory ducts above their entrance into the urethra and dorsal to the urethra below this point (post spermatic and post urethral). This is the part of the prostate which is felt per rectum. (4) Ventral lobe, or that part of the organ formed by glands arising from the anterior or ventral wall of the prostatic urethra.

¹⁸ Primrose, *Jour. of Anatomy and Physiology*, 1899, p. 64.

¹⁹ Minot, *Human embryology*, New York, p. 490.

²⁰ Keibel-Mall, *Human embryology*, Philadelphia, vol. 1, 1910, pp. 180-200.

*Fetus 5 cm. long.*²¹ (ten weeks)

A study of the bladder and prostatic portion of the urethra of a human male fetus two and one-half months of age shows several interesting features. The bladder at the trigonal region has about the same circumference as it has over the rest of its area, being at this stage a tubular structure which narrows down gradually as it approaches its orifice and nowhere is there a sharply outlined portion which will later become the vesical orifice. The prostatic portion of this tubular structure is marked only by the change in its shape.

The organs all seem to be composed of embryonic connective tissue which is similar throughout. The walls of the bladder are uniform in size everywhere except in the region of the trigone which is nearly twice as thick as any other portion. The connective tissue strands can be traced from the ureters out into the trigone and the latter structure is quite definitely superimposed upon the bladder wall. The increased thickness of the base of the vesical wall extends throughout the trigonal region but is most marked at the beginning or interureteral region.

By following this gradually narrowing tube down there is noticed a change in shape so that there is a slight notch formed in the ventral wall of the urethra and a projection into the lumen of the posterior wall so that this structure takes on the shape of a very widely spread inverted V. This marks the beginning of the verum montanum. Further down there are noticed two little notches on the floor of the urethra, one on each side of the verum montanum. The one on the right is more pronounced than that on the left. The outer or lateral portions of the lumen which will later become the prostatic furrows point in a horizontal direction and at this period of development show no tendency to be directed downward (fig. 1).

In no portion of the prostatic urethra is there any thickening of tissue or outgrowth of epithelial cells indicating the development of prostatic gland tissue. There is no specific arrangement of tissue planes and it is not possible to pick out the exact site of the

²¹ These measurements are all crown-rump and not crown-heel.

internal sphincter. Below the verum montanum the urethra becomes more or less star-shaped, indicating a collapsed circular tube.

Fetus 7.5 cm. long: (thirteen weeks)

There is considerable change noted in the appearance of the bladder at this stage. Throughout its entire area the wall composing the base is thicker than at any other portion of its circumference, and the nearer one approaches the trigonal region the greater is the thickness. The musculature of the bladder is dis-

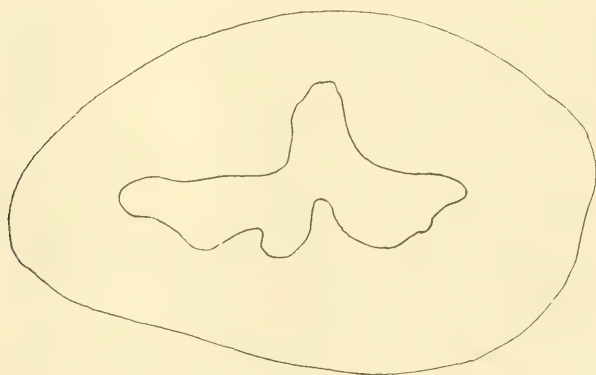


Fig. 1 5 cm. human fetus two and three-fourths months. Prostatic portion of urethra.

tingly made out as deeply staining tissue composed of circular, interlacing and longitudinal strands which are easily differentiated from the connective tissue elements forming the major portion of the bladder wall. The strands of muscular tissue are much larger and more abundant at the base or inferior portion than at any other. In the region just superior to the trigone where the bas-fond will later develop, the inferior wall is three times as thick as it is at any other part of the circumference. The mucous membrane is gathered in folds on the inferior interior surface of the organ throughout its length, while elsewhere it is smooth.

The trigonum vesicae is about five times as thick as the remaining portion of the vesical wall. The muscular strands composing

it are much finer in texture than those found elsewhere and many of them can be traced between the two ureters and out into other portions of the trigone.

There is a very sudden narrowing of the vesical walls at the site of the developing internal sphincter and the lumen of the bladder changes from an oval to a triangular shape and then into a rather narrow horizontal slit with a vertical slit connecting with its anterior wall as shown in fig. 2. Considerably below this it becomes triangular in shape again.

An examination of the urethra of the thirteen weeks old fetus from the bladder outwards reveals the fact that two large solid

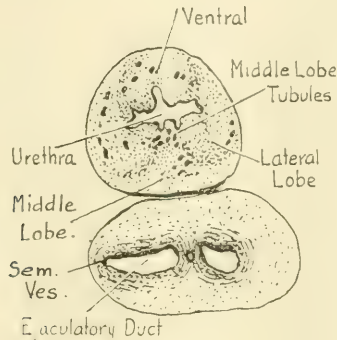


Fig. 2 7.5 cm. human fetus. Three months. $\times 20$.

evaginations extend posteriorly from its floor. Outward or caudally from these buds other larger evaginations have developed forming tubules, in some of which, lumina are present. In others the lumina are poorly developed, and in still others are solid (fig. 2). These structures, 12 in number, which are without question developing prostatic tubules, are separated by a considerable space from the main mass of prostatic tissue and occur directly on the floor of the urethra in a position that is universally accorded to the middle lobe, i.e., between the bladder and the entrance of the ejaculatory ducts, and extend posteriorly to occupy a position under the floor of the urethra and between the bladder and ejaculatory ducts.

The tubules of the lateral lobes arise from the sides of the urethra and from the bottom and in some cases a little to the inside of the depressions at the sides of the urethra, which are commonly called prostatic furrows. These structures are in nearly every case larger than the ones making up the middle lobe. They are thirty-nine in number, twenty-six of which are arranged definitely in pairs and three of which are unpaired send branches forward.

In this series no glandular tissue is noted in the region dorsal to the ejaculatory ducts. At the lower or caudal end of the prostate the tubules become centrally located near the midline and here we have structures which later grow back dorsally to the ejaculatory ducts and become the posterior lobe of the prostate. Beginning evaginations and tubules occur throughout the prostatic urethra on its roof or ventral wall. The general direction of the growth in this region as well as in the others is bladderwards with the exception of the outermost of the lateral lobes and the posterior lobe which send a few branches caudalward. The tubules of the ventral lobe are twelve in number, eight of which are paired, the other four being directly in the middle line. One of the latter tubules is quite long and presents a very definite lumen as all of the larger ones do; those smaller in size are nearly all solid epithelial outgrowths.

There are no glandular outgrowths from the urethra below the apex of the prostate and there are no signs of glandular growth in the subcervical region where Albarran's tubules are found in later stages.

The vasa deferentia appear under the bladder at the entrance of the ureters as two small tubes surrounded by a thick layer of developing muscle and connective tissue. As they descend they approach each other and behind the middle of the trigonum vesicae they are enveloped in the same tissue, being bound very firmly together, and between them is noticed a very small lumen surrounded by rather delicate but distinct connective tissue layers, which is taken to be the unobliterated upper portion of the fused Müllerian ducts. The vasa deferentia and their enveloping tissue increase enormously in size as they descend, so that under the internal sphincter this structure is larger than the beginning

of the urethra and its surrounding tissue. Under the main portion of the middle lobe the lumina of the vasa deferentia spread out laterally as shown in fig. 2 and form a lateral branch or bending which marks the first appearance of the seminal vesicles. Immediately below this point at the beginning of the ejaculatory ducts, the lumina constrict, and the surrounding tissue is less abundant. The structures surrounding the ejaculatory ducts which have to this point remained separate now intermingle with the muscular walls of the urethra and firmly bind the two structures together.

At this point the utriculus prostaticus which has been very small begins to enlarge and finally becomes larger than the two ejaculatory ducts which decrease in size as they approach the urethral lumen.

In the progress of the two ejaculatory ducts and utriculus prostaticus toward the lumen of the urethra, the tissues surrounding the two structures are closely bound together, but the mass of tissue around the ejaculatory ducts and utriculus prostaticus maintains its identity and by its further progress pushes the flattened floor of the urethra up into a mound-like projection transforming its triangular lumen into an inverted semilunar shaped structure and forming the verum montanum, the main tissue of which is thus derived from the walls of the ejaculatory ducts.

Fetus 8 cm. long: (thirteen weeks. No. 54 in Dr. Mall's collection)

This fetus shows exactly the same distribution of elements as the one just described. The number of tubules is reduced, probably due to the fact that the sections were three times as thick as the ones discussed above and this might have obscured some of them.

The middle lobe region gives rise to seven glands. Twenty-seven tubules are found in the two prostatic furrows which will form the lateral lobes and six others are located on the floor of the urethra caudalward from the entrance of the ejaculatory ducts which represent the posterior lobe of the prostate. These latter send branches caudalward or anteriorly as well as cranialward or

posteriorly, and none of them extend as far back as the ejaculatory ducts.

On the ventral wall of the urethra are located thirteen structures composing the anterior lobe.

The subcervical glands of Albarran were not found.

Fetus 12.5 cm. long: (sixteen weeks)

The bladder of the fetus studied here was contracted so that a direct comparison with the younger stages which were distended, is not possible. However, it is very evident that there has been an enormous development of smooth muscle between the last specimen described and this one. Throughout the whole vesical wall the muscle bundles making up the three coats of the bladder stand out very clearly. The mucous membrane and the connective tissue underlying it are thrown into thick folds extending into the lumen of the bladder and almost obliterating it. In this specimen as in those previously described the trigonum vesicae is very much thicker than any other part of the bladder wall. Muscle fibers are seen in large number extending from the ureteral walls and forming a thick layer which seems to be superimposed upon the true vesical wall as described by Poirier and Charpy.

The folds in the bladder mucosa disappear from the trigonal region, although they persist in other portions of the vesical lumen, until the region located at about the middle of the trigonum vesicae is reached where it is smooth and regular. In this same region the trigonal portion of the vesical wall is no thicker than any other portion, although lower down just before the commencement of the internal sphincter the trigonal portion is about twice as thick as any other part of the bladder wall.

Just below the internal sphincter which is quite well developed at this age there are noted eight very slight finger-like evaginations from the floor of the urethra. These structures extend only a short distance into the submucous tissue and will form the subcervical group of glands described by Albarran.

A very short distance below the lower part of the internal sphincter there are seen numerous gland tubules which are situated near the periphery of the greatly thickened urethral wall. These ends of prostatic tubules are arranged in four groups which are rather widely separated from one another by the stroma of the urethral wall. These groups of gland tubules are located one in each lateral wall, one between the floor of the urethra and ejaculatory ducts, and one in the anterior wall. Surrounding each tubule is noted a slight differentiation of tissue from that composing the wall of the urethra, which in this stage shows equal thickening on all sides, and there is not as yet any bulging, although the posterior wall is enlarged as it was in the 13 weeks fetus due to the presence of the ejaculatory ducts and the utriculus prostaticus with their surrounding tissue layers.

Careful identification of the various groups of tubules mentioned above and tracing their course from section to section reveals the fact that even at this early stage these structures have many branches which communicate with the urethra by means of one rather small duct. In one case there are three extensively branching tubules whose ducts join and empty into the urethra through a common duct (fig. 3).

In this series of sections the tubules are distinctly divided into five different groups as follows:

The middle lobe is composed of ten extensively branching tubules which are separated from the two lateral lobes by a rather thick layer of connective tissue. The branches of these tubules which are situated in the posterior wall of the prostatic urethra between the ejaculatory ducts and the bladder join and communicate with the urethra by means of ten ducts whose mouths are situated upon the floor of the urethra a considerable distance bladderward from the openings of the ejaculatory ducts and are grouped together in a very characteristic manner, being surrounded and bound together by connective tissue and developing smooth muscle fibers (fig. 4). The location of these tubules, their course as graphically shown in fig. 3 by means of a composite drawing which represents the change in location of the various

groups of tubules and not the individual branches, and the grouping of the duct openings of these tubules on the floor of the urethra as they communicate with it, demonstrate clearly that this structure is an independent part of the prostate gland.

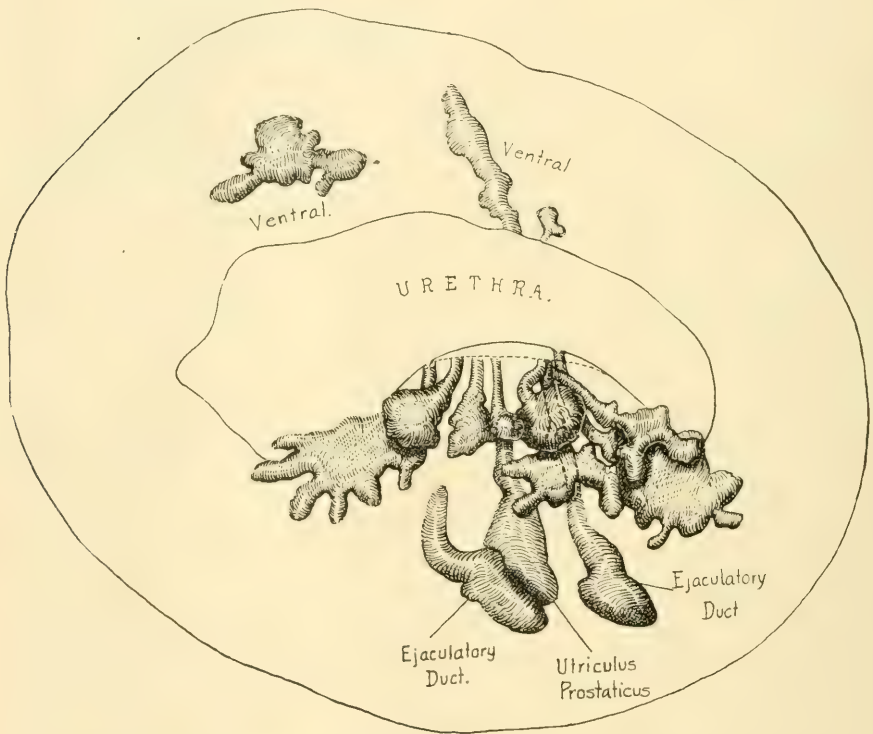


Fig. 3 Composite drawing showing course of tubules of the middle lobe of prostate; 12.5 cm. human fetus. Four months. $\times 30$.

The two lateral lobes are composed of tubules which are larger in size and branch more extensively than those of the middle lobe. The units composing these lobes grow posteriorly as well as laterally and occupy a region to the outside of the middle lobe and the ejaculatory ducts. In this specimen there are forty-six tubules composing the lateral lobes, the ducts of which communi-

cate with the urethra not only at its sides but also in the depressions on each side of the verum montanum commonly referred to as the prostatic furrows.

In the posterior wall of the urethra outerward from the entrance of the ejaculatory ducts and widely separated from all of the other lobes of the prostate, are found four large branching tubules which form the posterior lobe of the prostate. The branches of these tubules are not at any place in close touch with the lateral lobe tubules which extend this low in the urethral wall and there seems to be a definite layer of connective and muscular tissue forming around the component parts of this lobe.

Extending along the ventral or anterior wall of the prostatic urethra are observed the tubules forming the anterior lobe. Fourteen ducts open into the urethra in this specimen. Most of the tubules are quite small. Only two are as large and extensively branching as those of the other lobes already described.

Below the outermost tubule of the posterior lobe the urethra changes its shape from the inverted semilunar type shown in the drawings to a stellate shape. Extending from the walls on all sides are small finger-like evaginations. Some of these are simple folds in the urethral mucosa but others are developing tubules, some of which have extended quite deeply into the submucous tissue and a few have small branches. These are considered to be the developing urethral glands. While they are quite numerous just below the apex of the prostate, below that they are not found at all.

The seminal vesicles in this series are composed of very tortuous tubes which have a thick muscular layer surrounding them and communicate by means of one small duct or opening into each vasa deferentia under the internal sphincter.

The two ejaculatory ducts bound together as described above become attached to the wall of the urethra below the point just mentioned and gradually become more deeply implanted in the thick posterior urethral wall. The utriculus prostaticus is entirely obliterated above but appears between the ejaculatory ducts in the middle of the prostatic urethra. These three struc-

tures bound together by connective and muscular tissue in a very characteristic way approach the urethra on a gradual slant maintaining the globe-like appearance shown in fig. 4, pushing the floor of the urethra up into its lumen, forming the verum montanum and causing the urethra to assume an inverted semi-lunar appearance. The ejaculatory ducts run parallel to the floor of the urethra for a considerable distance and then ascend vertically to empty on the sides of the verum montanum. The

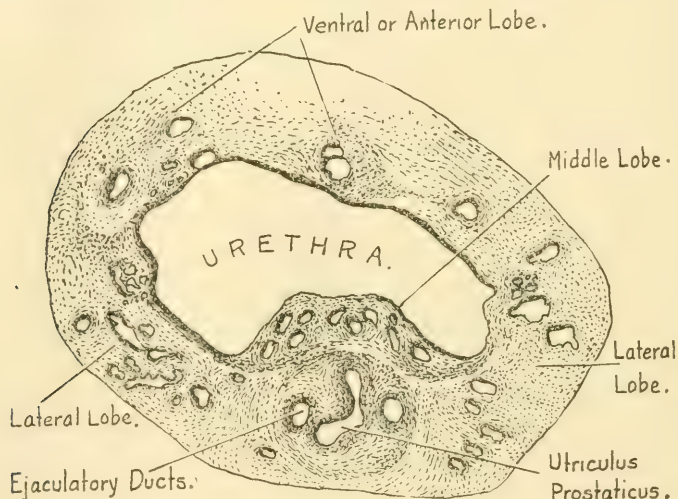


Fig. 4 12.5 cm. human fetus. Four months. Showing rather definite separation of the middle lobe from the lateral lobes.

utricle prostaticus in this series is very irregular in shape, being throughout most of its length in the shape of an inverted Y and giving the appearance of two tubes which have fused at their upper pole and not at the lower. Its opening into the urethra is below those of the ejaculatory ducts and there is a blind end extending a short distance down in the verum montanum under the floor of the urethra.

Fetus 12 cm. long: (sixteen weeks)

A series of sections cut in the longitudinal direction through the bladder and prostatic urethra of a fetus four months of age shows the arrangement of the various structures in a very striking manner. The smooth muscle of the bladder wall stains very deeply and is bound in place by the lighter staining connective tissue. The mucous membrane of the bladder and the submucous tissue is thrown into folds and finger-like projections into the vesical lumen except over the trigonum vesicae where it is tightly adherent to the muscle forming this structure. The trigonum vesicae is made up of muscular fibers which are finer in texture, more tightly bound together, and have lighter staining characteristics than the rather heavy, loose, deeply staining muscle bundles of the bladder wall proper upon which it is superimposed. The trigonal tissue extends down through the vesical neck and becomes lost among the muscle fibers of the prostatic urethra. The sphincter of the bladder appears as a large oval mass of circular fibers which surround the neck of the bladder and are quite distinct from the surrounding structures. There is a sharp line of differentiation around its lower part, while above some fibers from the trigone and vesical wall mingle with the fibers of the sphincter attaching it intimately to them.

The distribution of the tubules of the prostate gland is similar to that just described in the cross sections of the bladder and prostatic urethra of a fetus at this age. On the floor of the urethra just below the vesical sphincter several small evaginations represent the developing glands of Albarran. The middle lobe of the prostate is composed of several large tubules with a number of branches which extend back posterior to the sphincter and anterior to the ejaculatory ducts. The lateral lobes are somewhat larger than the middle lobe, being composed of numerous tubules with many large branches. The posterior lobe tubules are seen extending from the floor of the urethra below or outward from the entrance of the ejaculatory ducts to a position behind them. On the roof or ventral wall of the urethra are found the tubules

which make up the ventral lobe of the prostate. In this specimen they are not nearly so numerous or so large as in the series of cross sections just described.

Fetus 16.5 cm. long: (twenty weeks)

The bladder and prostate of a fetus five months of age cut in cross sections show the musculature of the bladder wall and the superimposed trigonum vesicae much more clearly than any of the previous specimens described. A striking thing is the great thickness of the vesical sphincter whose many layers of circular fibers form a mass about as thick as the bladder wall at its base. At its upper and also outer borders the muscle fibers of the bladder wall intermingle with it, thereby strengthening the vesical neck to a considerable degree. The vesical orifice in this specimen seems to be tightly closed. The muscular layers surrounding the tubules of the prostate gland whose earliest differentiation from the rest of the wall of the prostatic urethra was noted in the sixteen weeks fetus, have now become quite thick and the main mass of fibers are arranged circularly, although there is noted a certain amount of muscular tissue extending longitudinally as described by Walker.²²

The same distribution of the prostatic gland tubules is noted in this series of sections that has already been described in detail in the prostate of a fetus sixteen weeks old.

Fetus 16 cm. long: (twenty weeks)

Sections of the bladder and prostate of a fetus five months old cut in a longitudinal direction show particularly well the arrangement of the structures at the vesical neck. The muscle bundles of the bladder wall are well developed and arranged in the characteristic manner already described. The bladder mucosa which elsewhere is thrown into folds is bound tightly to the surface of the trigone which is superimposed upon the vesical wall and is composed of muscle bundles made up of very fine fibers which

²² Walker, Johns Hopkins Hospital Bulletin, vol. 11, p. 246.

do not stain so deeply as the muscle fibers elsewhere. The internal sphincter is sharply marked off and does not seem to be proportionally so large as the one observed in the twenty weeks fetus cut in cross sections.

Just under the mucosa of the trigonum vesicae is seen a small collection of simple tubules which are taken to be the subtrigonal glands (fig. 5). They are few in number and are very delicate in structure.

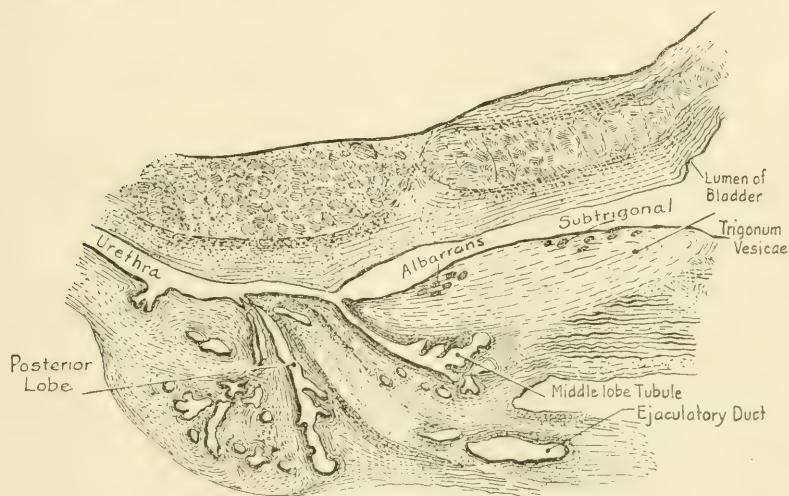


Fig. 5 16 cm. human fetus. Prostate. $\times 15$.

Below the internal sphincter another collection of glands is seen developing from the floor of the urethra. These have not as yet grown deeply into the wall of the prostatic urethra. They are identified as the subcervical glands of Albarran and in this series are very few in number and very slight as regards their architecture.

Extending from the floor of the urethra backward and downward is seen a tubule of the middle lobe of the prostate with numerous branches which extends into the region ascribed to the middle lobe and is posturethral and prespermatic. The ejaculatory duct is observed passing up into the verum montanum. Below its entrance there are several tubules of the posterior lobe

with many branches and whose ducts communicate with the floor of the outer portion of the prostatic urethra. The section which shows this arrangement of structures is reproduced diagrammatically in fig. 5. It was cut a little to one side of the middle line as shown by the presence of part of the ejaculatory duct and its muscular wall, and shows quite conclusively that tubules of the middle and posterior lobes arise independently from the urethra and in this instance could not be construed as extending in towards the middle line from the lateral lobes.

Other sections of this series show the tubules developing from the prostatic furrows and sides of the urethra to form the lateral lobes. Others extending from the ventral or anterior wall make up the anterior lobe of the organ. In this specimen these latter are quite numerous and are extensively branched.

Fetus 19 cm. long: (twenty-two weeks)

The bladder of this specimen is relaxed and the lumen almost filled with the loose folds of mucosa pulled into it by the contraction of the muscles of the wall, which are very large in this case compared to the specimens previously described. The three muscular layers are clearly made out and rather larger fibers than those heretofore described are seen passing from the wall of the entering ureters out upon the vesical wall, forming the trigonum vesicae. The upper border of this structure is about twice as thick as the bladder wall and more compactly arranged. Lower down the trigone is less thick and at its middle is not as thick as the bladder wall proper. The mucosa covering the trigonum vesicae is tightly bound to it so that in relaxation of the bladder the mucosa of the rest of that structure may be thrown into many large folds but the trigonal mucosa does not so arrange itself.

Extending parallel with the long axis of the lumen of the bladder just outside of the mucosa of the roof or ventral wall of that structure is seen a body which arises by two small branches and ends blindly in the vesical wall. It extends from the upper border to the middle of the trigonum vesicae and at its upper end the branches become indistinguishable from muscle bundles.

Throughout its lower portion after the two branches join it is surrounded by a thick layer of circularly arranged fibers enclosing this other structure, which in some places seems to be a lumen filled with degenerated epithelial cells and at others a space filled with bundles of fibers undergoing degeneration. This structure is about ten times as large as any of the blood vessels seen in the section and does not bear any resemblance to tissue seen in this or other series studied.

There are five small tubules seen developing from the floor of the bladder and extending down into the lower part of the trigone. These are the subtrigonal glands.

The circular fibers composing the sphincter at the vesical neck are quite thick in this case and are intimately connected with the muscle bundles of the bladder proper, while the trigonal fibers although they are very few in number down here are superimposed on the fibers composing the sphincter. This arrangement persists until the upper portion of the prostatic urethra is reached where they become lost in the musculature of the urethral wall.

Just below the vesical sphincter there are found eleven evaginations from the floor of the urethra which are lined with very fine cylindrical epithelium and which in no case branch. These tubules are recognized as the subcervical glands of Albarran and their direction of growth seems to be upward toward the bladder and in no instance do any of them extend deeply into the musculature of the urethra.

In the region usually occupied by the middle lobe tubules of the developing prostate gland, there is in this series an entire absence of such tubules (fig. 6). Very careful study fails to reveal any glandular tissue, except Albarran's tubules described above, developing from the floor of the urethra between the entrance of the ejaculatory ducts and the cervix of the bladder. Extending from each of the lateral lobes is seen a large branch from the tubule nearest the middle line, which, if it should continue to grow and send out additional branches, would ultimately form a bridge of tissue extending from one lateral lobe to the

other and binding them intimately together. This is the condition which Pallin believes to exist in all prostate glands.

The two lateral lobes are composed of 42 distinct tubules which have numerous and large branches. These structures all extend laterally, posteriorly, and towards the bladder, their uppermost branches being found under the lower end of the vesical sphincter. The muscular layers around the tubules, the development of which has already been referred to, are very prominent in this specimen, particularly where the tubules are beginning to push out laterally and posteriorly to form the bulging lateral lobes.

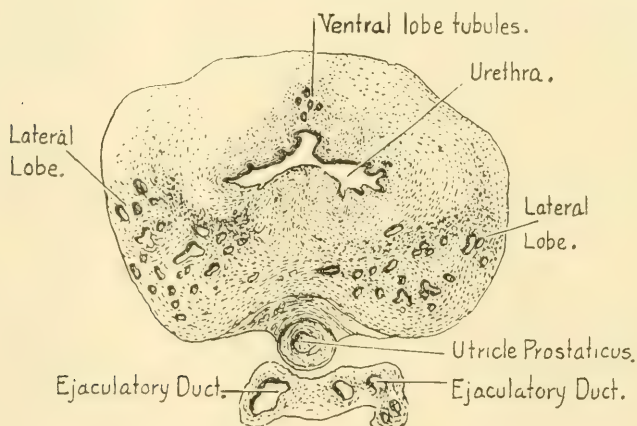


Fig. 6 19 cm. human fetus. Five and one-half months.

Below the entrance of the ejaculatory ducts and utriculus prostaticus there are observed ten large branching tubules which extend back behind the structures mentioned above and between and behind the lateral lobe tubules. These tubules compose the posterior lobe of the prostate and make up the main mass of its apex, being distinctly separated from the two lateral lobes by a considerable area of tissue. The posterior lobe does not extend very far up behind the ejaculatory ducts in this specimen on account of the huge size of the utriculus prostaticus which occupies the space into which the tubules of this portion of the gland usually extend.

The anterior lobe consists of seven tubules which are very much smaller than those making up the other lobes and very few branches are sent off. There seems to be a shrinking into insignificance of these tubules as far as a comparison with the size of similar structures in the younger fetuses is concerned.

The seminal vesicles are very tortuous in this series. The ejaculatory ducts seem to be of normal size but in their course through the wall of the urethra they are separated by an utriculus prostaticus, the upper solid end of which is seen in fig. 6. This structure is a little larger in size than the ejaculatory ducts in most of the specimens studied, but in this case it is about twenty times as large as an ejaculatory duct and at least three times as large as the lumen of the urethra. It is as usual contained within the same muscular sheath as the ejaculatory ducts as shown in fig. 4 but here the muscular coat is very much thinned out. The utricle extends obliquely through the urethral wall accompanied by the ejaculatory ducts until one part of its circumference is quite close to the floor of the urethra, where a small part about the size of a normal utricle separates from the main part of the organ and runs along under the floor of the urethra for some distance below the openings of the ejaculatory ducts. It then opens in the midline on the crest of the verum montanum. The larger part of the lumen runs forward on the same plane that it occupied above and stops rather sharply in a blind end. There have not been seen either prostatic or ejaculatory ducts opening into the utriculus prostaticus of this or any other specimen studied.

The ejaculatory ducts are observed to run obliquely through the prostate until they approach quite close to the urethra in the crest of the verum montanum; then they run parallel with the axis of the urethra for a considerable distance (300μ in this case), ultimately opening on a slant into the prostatic urethra on each side of the verum montanum, so that any pressure within the urethra would tend to close them very effectually and in a manner similar to that observed in the ureters in cases of distention of the bladder.

The verum montanum is formed in this case as already described by the ingrowth of the utriculus prostaticus and ejaculatory ducts,

Their development under the floor of the urethra pushes it up into the lumen causing the characteristic semilunar shape of the prostatic urethra. The fact that the lumen of the prostatic urethra below the entrance of the ejaculatory ducts is also crescentic in shape is due to the continuance of the muscular fibers that form the coats surrounding the utricle and ejaculatory ducts under the mucosa of the urethra where they finally become lost in the muscle of that structure.

Fetus 27 cm. long: (thirty weeks)

Inspection of the bladder of this specimen cut in cross section corroborates the findings already described, the only difference being that the muscle bundles seem to be larger in this than in any of those previously observed. The trigone is superimposed in the characteristic way and its submucous blood vessels, which in all cases are much more numerous than those of any other portion of the viscus, are greatly increased in number and size in this case. The sphincter of the bladder is thicker and composed of heavier muscle fibers than the ones that have been described. Outside of the sphincter are seen the muscle bundles of the vesical wall which become blended with and are lost in the circular fibers forming that structure.

The subtrigonal glands are present in very small numbers, only four being detected. They extend through the submucous structures and only a very short distance into the musculature of the trigonum vesicae. Their architecture is very slight and there seems to be no particular differentiation of connective tissue or muscle fibers around them. They resemble the urethral glands of Littré more than any other tissue and probably are a continuation upward of those structures.

The subcervical glands are present in this series, being nine in number. They are all quite small and no branches are noted in any of them. In addition to these nine tubules on the floor of the urethra there are six found on its roof or ventral wall. These tubules are similar in extent and architecture to the subcervical group and are apparently not a continuation upward of the ven-

tral lobe of the prostate as they are widely separated from that structure and are entirely different in their character from prostatic tubules. All of these gland tubules extend from the urethra upwards or towards the bladder under the mucosa, and in no case do they extend deeply into the muscular tissue. They push out into the submucous tissue between the numerous blood vessels in this region and are not surrounded by a specially arranged musculature. The lumina of these tubules are about three times the size of the blood vessels found here, but are much smaller than the prostatic gland tubules.

The middle lobe is made up of eleven tubules, whose blind ends extend backward and upward under the sphincter of the bladder, each of which has a larger number of branches and communicates with the urethra through quite small ducts which open upon its floor some distance above the openings of the ejaculatory ducts and utriculus prostaticus. In the specimen studied previously there has been a wide and distinct separation of the middle lobe from the two lateral lobes. This distinctiveness is well shown in the fetus 16 weeks old (fig. 4). In this specimen it is easy to distinguish the middle lobe from its location, the direction in which the tubules extend, and the fact that its ducts communicate with the urethra at the same point that ducts of middle lobe tubules have in younger series. On the other hand, the layer of tissue separating the middle from the lateral lobes is not very extensive except at the base. There is no semblance of a special capsule and in some places the tubules of the middle lobe are side by side with the tubules of the lateral lobe, but in no place is there found an intermingling of the branches of one lobe with those of another. At the base of the prostate, up under the vesical sphincter where the prostatic tubules have grown farthest away from their point of origin, the separation between the middle lobe and the two lateral lobes is quite wide and there is a great deal of connective and muscular tissue between them. In no case has it been possible to find any difference between the architecture of the tubules of the different lobes with the exception that those of the middle lobe are usually not quite so large as the lateral lobe tubules. Finer histological studies of the glandular epithelium have not

always been possible, as some of the tissue is quite old and contraction of the epithelium has taken place.

The two lateral lobes are made up of thirty-six tubules which extend posteriorly and up under the internal sphincter as far as do those of the middle lobe. At the sides of the lower part of the sphincter there is a distinct bulging due to the further development of the lateral lobe tubules, which with the middle lobe form the base of the gland. This posterior and lateral bulging is noted throughout the entire prostatic region, although the larger number of tubules are massed in the base of the gland. There are a number of tubules whose ducts open into the sides of the urethra and which send branches up towards the ventral wall. These structures have many branches but they are much smaller than either the rest of the lateral or middle lobe tubules and their musculature is very slight compared to that of the other prostatic tubules. In the apex of the gland the tubules of the lateral lobe have a few branches which run downward or outward. Everywhere else the general direction of growth is towards the bladder.

The glandular tissue making up the posterior lobe is found at the point where the ejaculatory ducts extend vertically towards the urethra. It is between and behind the lateral lobes and this part of the gland is distinctly separated from the lateral lobes by a thick layer of connective tissue (fig. 7). This lobe in the series under consideration is made up of nine branching tubules of large size whose ducts communicate with the urethra on its floor or posterior wall outward from the openings of the ejaculatory ducts and at no point do any of the branches of these tubules intermingle with the tubules of the lateral lobes, being in all places separated from them by a definite layer of connective tissue. In the apex of the gland some of these tubules send branches forward or outward but elsewhere they extend in a bladderward direction.

The comparative decrease in size of the tubules of the anterior lobe noted in the prostate of the twenty-two weeks old fetus is also striking in this series. The number of tubules making up the anterior lobe noted in the fetuses younger than twenty-two weeks was about twice as great as that noted in the specimens twenty-

two weeks and older. In this prostate there are eight small branching tubules communicating with the anterior urethral wall. They are surrounded by muscular layers similar to the other prostatic tubules and are quite limited in their extent and widely separated from the lateral lobes.

Below the apex of the prostate there are noted a large number of glands, most of which are simple tubules, extending into the sub-

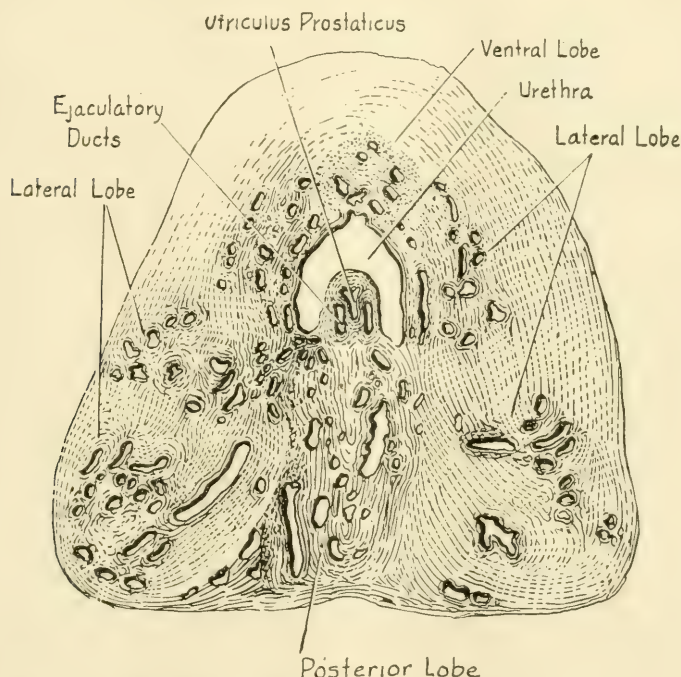


Fig. 7 27 cm. human fetus. Seven and one-half months. $\times 14$.

mucous tissue their ducts opening into the urethra. They are found on all sides of its lumen but most of them open on the ventral wall. They extend barely to the muscular bundles and none of them are found within the musculature.

The seminal vesicles have grown back behind the base of the bladder to a point opposite the middle of the trigonum vesicae. They are made up of thick walled tortuous connected tubes which

communicate with the vasa deferentia behind the lower part of the sphincter of the bladder rather deeply in the base of the prostate gland.

The ejaculatory ducts surrounded by their thick muscular wall and imbedded in the prostate traverse its posterior portion for a considerable distance in a plane horizontal with the floor of the urethra. A little below the point where the lowest of the middle lobe tubules opens out upon the floor of the urethra the ejaculatory ducts turn sharply and mount almost perpendicularly towards the urethra and with their thick coats and the utriculus prostaticus form the verum montanum. By referring to fig. 4 it is seen that the relative sizes of the urethra, the verum montanum and its contained structures are much smaller in the older specimen when compared with the size of the prostate gland. The ejaculatory ducts, which are greatly narrowed here, run for a considerable distance in the verum montanum parallel with the axis of the urethra and finally open on the sides of the verum montanum. The course of the ducts in this compressible structure and their lateral openings which are very small in size, are the features which make total occlusion of these important structures possible in case of distention of the prostatic urethra from any cause.

The utriculus prostaticus is about the size of an ejaculatory duct in this fetus. Its uppermost end begins at the point where the ejaculatory ducts assume their position in the verum montanum and extends outward in the apex of that structure opening in the middle line near the apex of the prostatic urethra. Outward from the mouths of the ejaculatory ducts the utricle is surrounded by a thick muscular wall which includes a continuation of the fibers which surrounded the ejaculatory ducts. Below the mouth of the utricle the muscular tissue continues for a short distance only.

New-born infant: (35 cm.)

The bladder and prostate of an infant who had died a few days after birth were cut in cross section and mounted in the usual way, being stained with haematoxylin and eosin. A very thorough study has been made of the prostate of this baby, which has in-

cluded drawings, diagrams, and the construction of a wax model, pictures of which are reproduced in this article.

The bladder was distended upon fixation and the natural curve at its neck mechanically straightened, so it is possible to get a good idea of the thickness of its walls at various points. At the place where the ureters join the vesical wall and begin their oblique course through to its lumen, there is an increase in the size of the base to twice that observed elsewhere in its circumference. This increased thickness of the base is maintained throughout the trigonal region, being most pronounced between the ureters where the interureteric ridge is made up of fibers extending from one ureteral wall mingling with those of the other. The trigone in this as in the previously described cases is made up of muscular fibers which originate in the walls of the ureters and pass over the musculature proper of the bladder, becoming lost among the fibers of the urethra. Just below the interureteric ridge the wall of the base of the bladder becomes comparatively smaller, being very little larger than the rest of the wall. As the sphincter is approached the entire vesical wall increases greatly in size, the base always being considerably larger than any other part and the trigonal fibers are always distinguishable from the tissue of the vesical wall proper. The mucosa over the trigone is much more richly supplied with blood vessels than any other portion.

The internal sphincter is made up of circular fibers which are very numerous at its upper part. It is larger at the floor of the vesical cervix, and while many of the fibers pass entirely around the orifice of the urethra the majority of them mingle with the muscle bundles of the bladder on its ventral aspect which approach much closer to the lumen of the orifice of the urethra here than in the posterior wall. At the outermost portion of the sphincter there is a great decrease in the number of fibers composing it and it is about equal in size at all points of its circumference. In its ventral portion a number of small longitudinally disposed fibers are seen. The lower part of the sphincter ends considerably outerward from the point where posterior lobe tubules have extended backward behind the two ejaculatory ducts.

In the mucosa at about the middle of the trigonum vesicae are found nine very delicately constructed tubules which are recognized as the subtrigonal glands. Most of them are simple tubules that extend down to the muscle but a few of them have one or two very small branches and these extend for a short distance into the musculature of the bladder wall. The blind ends of these tubules are a little closer to the base of the trigone than the mouths of their tubules.

Commencing at about the middle of the vesical sphincter and extending down to its lower border there are found on the floor of the orifice the tubules forming the glands of Albarran. There are nineteen of these structures in this series, most of which are simple tubular glands lined with very small columnar epithelium and do not extend very far beneath the mucosa. In a few cases there are several branches springing from a tubule and these in many instances extend a short distance into the musculature of the sphincter. None of these tubules are found in the ventral mucosa of the orifice but there are some very small evaginations in that region which may later develop into tubules of the same sort. The blood vessels in the mucosa of this region are quite numerous and large. The tubules of Albarran's group are not surrounded by any differentiated tissue as are the tubules of the prostate but seem to be merely imbedded in the submucous structures. They open for the most part near the middle line on the floor of the urethra but a few open in the angular depressions at the sides of the urethra which marks the beginning of the prostatic furrows.

The middle lobe tubules have extended up behind the sphincter to its uppermost border. The end of the tubule that has extended the highest is situated in the middle line just above the ampulla of the vas and its branches are surrounded by rather dense muscular tissue. The whole mass lies imbedded in the loose connective tissue beneath the bladder musculature. Lower down in the series these branches are reinforced by others of a like nature and a very short distance below become connected with the musculature surrounding the urethra. The tissues which envelop the middle lobe tubules are thicker in this specimen than in any of the younger ones observed being in places as dense as the walls

of the ejaculatory ducts. A few of the most lateralward of the middle lobe tubules have branches which extend to the sides for short distances but in no place is there noted an intermingling with lateral lobe tubules. There are no branches of the lateral lobes extending into the middle lobe region and the ducts of the nine large branching tubules which form the latter structure pour their secretions through mouths which empty upon the urethral floor bladderward from the openings of the ejaculatory ducts. An interesting thing noted in all of the prostates studied but more particularly in this one is the fact that an enormous number of branches join together to form one tubule which empties into the urethra through a duct which is no larger in diameter than one of its smallest branches. The ducts of the middle lobe tubules just before their entrance into the urethra are quite widely separated from other parts of the prostate, thereby retaining their embryological characteristic of an independent origin.

The uppermost ends of the left lateral lobe tubules have extended back under the bladder and are found contained in their thick muscular envelope adherent to the sides of the seminal vesicles. The right lateral lobe tubules are found to have extended back only to a point above the opening of the seminal vesicle into the ejaculatory duct. Lower down where the prostate is broadest the branches of the lateral lobe tubules are exceedingly numerous. By their great development they cause the base of the prostate to bulge laterally and posteriorly. The two lobes are made up of thirty-four tubules and are separated posteriorly by the ejaculatory ducts, the middle lobe and the posterior lobe. Anteriorly in this series of sections the branches of the lateral lobes approach each other very closely, especially in lower part of the gland at its apex. The outermost or caudalward portion of the lateral lobes, represented by two large gland tubules, send branches in a caudalward direction and this fact is of interest surgically because in nearly every successful enucleation these forward branches must be cut with curved scissors in order to free the lobes from the capsule as they seem to be particularly adherent. The posterior borders of these lobes are separated from the posterior lobe by a rather dense layer of connective tissue

which also separates it from the middle lobe and ejaculatory ducts (fig. 8). An increase in the amount of connective tissue at this point would be of great interest surgically in cases of perineal prostatectomy, because the incision must be made entirely through it, otherwise the enucleation procedure would lead the instrument to the capsule instead of to the inside of that structure and hence could not be completed.

The posterior lobe is made up of eleven glands, some of which have extended toward the bladder until they are almost as far

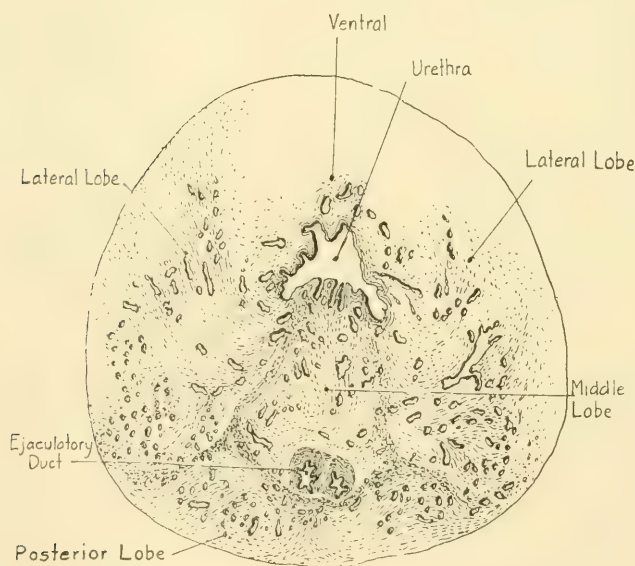


Fig. 8 36 cm. (new-born) baby prostate $\times 6$, camera lucida.

back as the ends of the middle lobe tubules. Their course follows rather closely the dorsal aspect of the ejaculatory ducts until the latter structures ascend almost vertically towards the urethra, immediately caudalward to which there is a small area free from glandular elements. The ducts of posterior lobe tubules enter the floor of the urethra caudalwards from the entrance of the ejaculatory ducts. The posterior lobe is the part of the prostate that is palpated per rectum and presents in the middle of its posterior

surface a slight depression which is ordinarily termed the median furrow. This depression in the specimen under discussion is more pronounced in the region of the apex and gradually becomes shallow at the middle of the gland and at the base assumes a rounded contour. At the apex two of the posterior lobe tubules send branches forward which appear outside of the muscular walls of the urethra, which at this stage are quite well developed.

The ventral lobe is composed of two very small tubules with just a few branches whose ducts open upon the ventral wall of the prostatic urethra at about its middle part. This lobe in the new-born has atrophied to almost complete insignificance (fig. 10).

Just at the apex of the prostate and a little below that point there are noticed numerous gland tubules contained in the sub-mucous tissue of the urethra which are easily differentiated from prostatic tubules, being much smaller in size and lacking the muscular coats of the latter. In most instances these tubules have several branches, but none of them are at all extensive as are the tubules of the prostate. The entire structures are contained within the muscular walls of the urethra and their ducts open into it on all sides. These tubules are considered to be the glands of Littré, and while very numerous just below the apex of the prostate are very few in number lower down in the urethra.

In the new-born the seminal vesicles have extended back under the bladder almost to the base of the trigone. Its uppermost portion consists of five lumina on each side with walls almost as thick as those of the vasa deferentia. These lumina all communicate lower down and connect with the ejaculatory ducts just below the point where they become imbedded in the musculature of the prostate gland. The ampullae of the vasa deferentia are easily distinguishable in this specimen and are marked by a considerable widening and great increase in the size of the lumen.

As the ejaculatory ducts become more deeply imbedded in the prostate they become situated in more immediate contact with one another until their musculature becomes intermingled as shown in fig. 8, and while each contains its own walls intact they are both contained within the same bundle. In their progress through the prostate they take the course already described in

previous specimens and which is shown in fig. 10. In this as in the other specimens studied the verum montanum is observed to be composed of the ejaculatory ducts, utriculus prostaticus and their walls. The ejaculatory ducts run for a shorter distance in the verum montanum in this than in the prostates previously discussed, finally opening on the lateral walls of that structure in the characteristic way already described.

The utriculus prostaticus is found in the tip of the verum montanum extending only a short distance before it opens into the urethra in the midline, and in this case has a very large wide opening above the mouths of the ejaculatory ducts which is not the usual arrangement, as in all of the other specimens studied its mouth has been below those of the ejaculatory ducts. There have not been found in this or in any of the other prostates studied either an ejaculatory duct or a prostatic tubule opening into the utriculus prostaticus.

DISCUSSION

1. The bladder of a fetus ten weeks old consists of a cylindrical tube composed of embryonic connective tissue. Near its base it is joined by the two ureters which pass through its wall and fibers from which are superimposed upon the bladder wall to form the trigonum vesicae. There is no tissue resembling muscle present and the future site of the vesical sphincter is not distinguishable except by the change in the size and shape of the lumen.

At the thirteenth week the muscular development has begun and is observed as circular interlacing and longitudinal strands of tissue that take on a deeper pink color than does the connective tissue which still forms the major portion of the bladder wall. The entire base of the bladder is thicker than any other portion of its circumference while the superimposed trigonum vesicae and the wall under it are five times as thick as the anterior wall. The muscle fibers observed in the trigonum are traced between the two ureters and down on the rest of the trigone. They are finer in texture than the muscle fibers in the bladder wall. The site of the internal sphincter is marked by a sudden narrowing in

the size of the lumen and a few circularly arranged muscular fibers are made out.

By the sixteenth week there has been a very great development of the musculature of the bladder, so that the fibers are sharply defined from connective tissue. The muscle fibers forming the trigonum vesicae are easily traced out into that structure from the ureteral walls and the mucosa which is arranged in folds everywhere else in this specimen is attached tightly to the trigone and is smooth. The sphincter vesicae has become quite sharply outlined by many muscular fibers arranged circularly at the constricted lumen of the cervix.

At the twentieth week the muscular fibers have become very much more prominent but the most striking change is the enormous increase in the size of and the number of fibers forming the internal sphincter which seems to be tightly closing the vesical orifice. The longitudinal fibers of the bladder intermingle with the outermost of the fibers of the sphincter. The mucosa in this stage is smooth over the trigonum vesicae and folded elsewhere.

In the twenty-two weeks old fetus there is noted still greater increase in the size of the bladder musculature and a marked increase in the size of the interureteric bar. The trigone and the bladder wall under it do not show the great increase in size over the rest of the vesical wall observed in younger fetuses. The fibers forming the trigone are observed to be more tightly bound together than the fibers of the bladder wall and the mucosa is smooth over it. The sphincter is large and closely bound to the rest of bladder musculature.

Further increase in the size of muscles forming the vesical wall, trigonum and sphincter is noted at the thirtieth week and in the bladder of the new-born. The sphincter in the latter is larger at its upper margin than elsewhere and is very thick on its posterior quadrant. Many of the fibers appearing there do not entirely encircle the orifice but intermingle with longitudinally arranged fibers from the anterior surface of the bladder. At the lower or outermost part of the sphincter there are fewer fibers but they all entirely encircle the orifice.

2. There is found in some of the sections studied a small group of glands which open upon the mucosa at the middle and lower part of the trigonum vesicae and which barely extend into the underlying musculature. These glands are in nearly every case simple tubules of very delicate structure, no muscular or specialized tissue layers being found surrounding them. They are not found until the twentieth week but are observed in every fetus older than that and also in the new-born. These tubules form the subtrigonal group of glands and are in every case few in number, nine being the most found and four the fewest. They are apparently insignificant but occupy a strategic position as a very slight hypertrophy at this point might cause a considerable obstruction to urinary outflow. Cases have been observed by Dr. H. H. Young and Dr. J. T. Geraghty in which this group of tubules had become enlarged and a further growth had caused them to become almost free in the bladder lumen being connected to the original site by a small pedicle. Upon attempted urination this globular mass would fall into the orifice of the urethra and blocking it would cause more obstruction than an enormous hypertrophied prostate. The structures are probably a continuation upward of urethral glands.

3. On the floor of the prostatic urethra of the fetus sixteen weeks of age at its commencement there are found eight small evaginations which are observed to extend only a short distance into the submucosa. These tubules are easily distinguishable from prostatic gland tubules being of very slight architecture and lacking the muscular layers which surround the prostatic tubules. This group of tubules first described by Albarran is found in all of the specimens studied here older than sixteen weeks. In fetal life they have no branches at all but in the new-born a few very small branches were made out. In all cases these tubules grow back toward the bladder in the submucosa and never extend deeply into the musculature. In the new-born they have grown back within the sphincter. These tubules are found in one instance growing from the roof of the prostatic urethra. They are few in number, varying from eight to nineteen and are very similar in structure to the subtrigonal glands just described, except

they are somewhat larger. The position of the sub-cervical glands of Albarran is even more strategic than that of the subtrigonal group. Growing back directly within the sphincter it is easy to see that a slight increase in size would form a very considerable obstruction to the passage of urine from the bladder.

4. The prostate gland begins to develop at the third month of fetal life. The tubules which compose it make their first appearance as solid epithelial outgrowths from five distinct parts of the prostatic urethra. These solid masses of deeply staining cells very soon become circularly arranged around lumen and branches are found very early. The five foci from which groups of prostatic tubules take their origin are located as follows: on the floor of the urethra between the neck of the bladder and the openings of the ejaculatory ducts and utriculus prostaticus, one in each prostatic furrow and on the sides of the urethra, on the floor of the urethra below the openings of the ejaculatory ducts and the utricle, and on the ventral or anterior wall of the prostatic urethra. The tubules originating from these five foci by their further growth and the development of stroma around them become the middle, right and left lateral, posterior, and anterior lobes respectively. In early fetal life they are widely separated from one another but in later stages the separation between the middle and two lateral lobes is not very great. There is not an intermingling of tubules in any of the specimens studied but in many places in the new-born the tubules of the middle lobe are observed side by side with those of the lateral lobes, there being no definite capsule separating them. The separation of the posterior lobe from the others is complete, as there is a rather dense layer of fibrous tissue between it and the lateral lobes. The anterior lobe is widely separated from the two lateral lobes.

The first appearance of muscular fibers developing around the tubules of the prostate is found at the sixteenth week at which time a slight differentiation in staining properties is noticed. At the twenty-second week the muscular layers are quite well developed and are particularly noted where some of the lateral lobe tubules have extended away from the base of the prostate. The musculature continues to become thicker and thicker until at

birth some of the tubules are surrounded by very dense muscular layers.

In every case but one in this study the middle lobe of the prostate develops independently from tubules which originate from the floor of the urethra near the middle line between the bladder and the entrance of the ejaculatory ducts. The tubules composing this lobe are separated from the lateral lobes by a considerable area of tissue free from glandular elements, the younger the embryo the greater the separation. In later development while the tubules and their branches maintain their characteristic position, there does not seem to be any definite formation of a fibrous capsule separating the middle from the lateral lobes. The ducts of the largest of the middle lobe tubules which were originally near the middle line are pushed laterally by the further development of the structures in the verum montanum so that they open rather high up on its sides and not in the middle line, as that part is occupied by the utriculus prostaticus and the ejaculatory ducts. Rarely (once in our experience) there may be an absence of independent middle lobe tubules, in which case branches from the lateral lobes are seen approaching the middle line in the region ordinarily occupied by the middle lobe, and if the growth continues this region will be occupied by a glandular commissure such as Jores declares all middle lobes to be. In most cases the middle lobe is made up of nine or ten large tubules, the number in five cases varying from seven to twelve, the average being ten.

As one of our embryonic series showed an absence of tubules in the middle lobe region it was deemed advisable to determine the percentage of prostates that have a middle lobe.

Dissecting room and autopsy subjects to the number of twenty were examined and all demonstrated definite prostatic tissue in the middle lobe region. Ten sets of serial sections of embryonic tissue demonstrated only one case without independent middle lobe tubules. Thirty-three autopsy cases of enlarged prostate in the Johns Hopkins Hospital Genito-Urinary Museum were examined, with the result that in thirty-one, definite middle lobes were identified. The existence of middle lobes in the other two cases could not be determined, as the specimens were not sec-

tioned. Two of the specimens examined had enlarged anterior lobes. Forty autopsy specimens of prostate gland enlargement were examined in the pathological museum at Guy's Hospital, London, thirty-seven of them showed the existence of glandular substance in the middle lobe region, while in three cases there was no macroscopic evidence of such a structure.

TABLE 1
Showing the frequency of the occurrence of middle lobes

SPECIMENS	NUMBER OF DEFINITE MIDDLE LOBES	QUESTIONABLE MIDDLE LOBES	MIDDLE LOBE DEFINITELY ABSENT
Twenty cadavers in Dr. Mall's laboratory.....	20	0	0
Ten fetuses.....	9	0	1
Thirty-three enlarged prostates, Dr. Young's clinic.....	31	2	0
Forty autopsy specimens of enlarged prostates, Guy's Hospital, London.....	37	3	0
Total.....	97	5	1

Reference to table 1 shows that there is a definite absence of glandular tissue in the middle lobe region in one specimen out of one hundred and three studied, and five specimens in which it was not determined whether there was glandular tissue there or not.

The lateral lobes are composed of tubules which are greater in size and number than those of any of the other lobes. They originate from the right and left prostatic furrows and the lower parts of the lateral walls and extend backward and outward forming the main part of the base of the prostate. In the younger specimens these lobes are widely separated from each other and the remaining lobes but the older the fetus studied the less is the separation between the branches of the lateral and middle lobes, although there is in all cases a definite separation observed where the ducts communicate with the urethra. In the region of the apex some of the lateral lobe tubules send branches forward, at all other parts of the gland the direction of the growth of tubules is back towards the bladder.

In one specimen there was observed a growth of branches from lateral lobe tubules into the region usually occupied by the middle lobe, the latter structure in this case being absent.

In the prostate of the new-born near the apex the lateral lobes have two extensively branching tubules which extend up into the anterior commissure and practically form a glandular commissure in this region.

The lateral lobes are in most cases very well separated from each other anteriorly a considerable area of stroma being present, in which are found the anterior lobe tubules. Mesially the ejaculatory ducts, urethra and middle lobe are interposed and there is a definite plane of connective tissue which sharply separates their posterior branches from the posterior lobe.

The number of tubules forming the two lateral lobes vary from twenty-seven to forty-six, the average number being thirty-seven.

The posterior lobe is made up of tubules which begin to develop with the other prostatic tubules at the third month. They are found on the floor of the urethra below the openings of the ejaculatory ducts and their direction of growth is behind those structures back towards the bladder. They compose the main mass of the apex of the gland and the posterior lobe is that part of the gland which is palpated per rectum. Just anterior to this lobe is found in the older stages a stroma of connective tissue free from tubules which separates it from the posterior parts of the lateral lobes and from the ejaculatory ducts. The tubules of this part of the prostate send a few branches forward in the region of the apex as do the most anteriorly arranged branches of the lateral lobes. They are quite large with numerous branches but are not very numerous, the smallest number being four, the greatest eleven, and the average in six specimens is eight.

This lobe is present in all fetuses here studied and in the new-born. It is an independent structure developing from tubules which are separated from those composing the other lobes and being divided from them by a definite capsule which is laterally connected with the capsule of the gland.

The posterior lobe is of considerable importance for several reasons. In doing Young's operation for perineal prostatectomy

operators have found it absolutely necessary to make their two parallel incisions quite deep so that they go completely through the connective tissue layer separating the posterior from the two lateral lobes. In case the incision is only made through the capsule of the gland into the posterior lobe, an attempted enucleation leads the operator's instrument or finger laterally into the outer capsule again where the anterior capsule of the posterior lobe becomes lost in it and enucleation of the real offenders in hypertrophy, lateral and middle lobes, is not possible until the incision is made into the capsule containing them.

Recent studies by Dr. John T. Geraghty and Dr. Montague L. Boyd on the pathology of the prostate gland have confirmed the facts (1) that hypertrophy rarely or never occurs in this lobe, (2) that primary carcinoma of the prostate usually arises in it. This knowledge coupled with the embryological fact that the tubules forming this structure arise independently from a localized area in the urethra and remain independent throughout, increasing enormously in size in the normal adult prostate, suggests the possibility that it may have a different function from the other parts of the gland.

The tubules forming the anterior or ventral lobe begin to develop at the same time as do those of the other lobes. They are large and have numerous branches at first, but in the sixteenth week they are slightly smaller than the tubules of the other lobes. At the twenty-second week these tubules have decreased in size and number and very few branches are noted. There seems to have been a shrinking into insignificance of the anterior lobe after the sixteenth week, but the tubules persist until birth, at which time there are found two very small tubules. We have evidence of the fact that the anterior lobe may persist throughout life in that among the ninety-three specimens of the adult prostate examined two were found with hypertrophied anterior lobes. The average number of anterior lobe tubules in the first half of fetal life is thirteen while that of the last half is six. Two tubules in the new-born were the fewest found, fourteen in the sixteen weeks old fetus being the greatest number.

In most descriptions of the prostatic urethra it is stated that there are from twenty to thirty duct openings upon its floor. My studies have convinced me that this number is far too low. The results of microscopic studies of the number of tubules composing the prostate have been arranged in the form of a table.

TABLE 2

Showing number of tubules of each lobe opening into prostatic urethra, the number of Albarran's tubules, and the number of subtrigonal tubules.

SIZE OF FETUS CROWN-RUMP MEASURE- MENT	MIDDLE LOBE	LATERAL LOBES	POSTERIOR LOBE	ANTERIOR LOBE	TOTAL NO. OF PROSTA- TIC TUBULES	SUBCERVI- CAL GLANDS OF ALBAR- RAN	SUBTRIGONAL GLANDS
<i>cm.</i>							
7.5.....	12	39	11	12	74	0	0
8.....	7	27	6	13	53	0	0
12.5.....	10	46	4	14	74	8	0
19.....	0	42	10	7	59	11	5
27.....	11	36	9	8	64	9	4
36.....	9	34	11	2	56	19	9
Averages*	10	37	8	9	63	12	6

* The averages are taken from the specimens in which the structure is present in case of middle lobe and the groups of Albarran and the subtrigonal group.

By referring to table 2 it is seen that in no case were there fewer than fifty-three prostatic ducts opening into the urethra, and in two specimens there were as many as seventy-four, the average for six specimens studied microscopically in series being sixty-three, including one specimen in which the middle lobe was entirely lacking. The number of middle lobe tubules vary from seven to twelve, the average in five prostates being ten. The lateral lobe tubules vary in number from twenty-seven to forty-six, the average in six specimens being thirty-seven. The posterior lobes show a variation of from four to eleven, eight being the average number of tubules in the six specimens recorded. The anterior lobes present a very interesting variation. It is seen that up until and including the sixteenth week the tubules composing the anterior lobe are quite large and numerous, but after that time there is a decided decrease in the number, and, as has already been stated, the size and branches of the individual

tubules. The greatest change in this structure is noted in the new-born in which the anterior lobe is made up of two insignificant tubules situated on the anterior part of the gland at its middle as shown in fig. 10.

The subcervical glands of Albarran are seen to occur in the specimen sixteen weeks of age and in all of the older ones. Their number varies from eight to nineteen, the average in the four series being twelve.

The subtrigonal glands are not found until the twenty-second week and they are in all cases few in number, varying from four to nine, the average in three specimens being six.

5. The vasa deferentia descend behind the posterior surface of the bladder, each one being situated near the ureter. Lower down they gradually approach one another and in the earlier stages become contiguous behind the middle of the trigonum vesicae. In their descent they increase enormously in size, so that under the internal sphincter of the bladder they with their enveloping tissue are larger than the commencement of the urethra with its surrounding tissue. At this point the lumen of the vasa deferentia have widened considerably denoting the first appearance of the ampullae. These structures remain comparatively large until the sixteenth week after which they become relatively very much smaller.

The seminal vesicles originate in the thirteenth week. They appear first as an evagination lateralward from each vasa deferens being covered by the same tissue that envelops the latter structures. They grow backwards and laterally consisting of a main part which is convoluted and from which rather numerous, short, convoluted branches grow out as described by Pallin.²³ In the further development of these structures the opening into the ejaculatory ducts becomes comparatively smaller and its component parts become larger and more tortuous. In the thirty weeks old fetus they are found back under the trigonum vesicae at about its middle point and communicate with the vasa deferentia deeply in the base of the prostate to form the ejaculatory ducts. At

²³ Gustaf Pallin, *Archiv für Anatomie und Physiologie*, 1901.

birth the ends of the seminal vesicles have extended back almost as far as the base of the trigonum vesicae and four branches from the main lumen of the organ are made out. Its walls are made up of fibrous and muscular strands and are almost as thick as those of the ejaculatory ducts.

The ejaculatory ducts are the continuations of the vasa deferentia below the entrance of the seminal vesicles. They are like the vasa deferentia very large in size in the younger fetuses but gradually become comparatively smaller in size as the other structures grow larger. They pass obliquely through the posterior wall of the prostatic urethra accompanied by the utricule and the surrounding stroma layers the outer fibers of which attach this cylindrical mass intimately to the urethral wall by intermingling with the tissue forming it. As these structures approach the lumen of the urethra its floor is pushed up into a mound and its lumen converted from a triangular into a semilunar shape. This mound, called the verum montanum, is made up entirely of the ejaculatory ducts, utricule and surrounding envelopes, and gradually disappears below the openings of these several structures into the urethra, the stroma cells becoming intermingled with those forming the urethral wall.

The ejaculatory ducts pass through the posterior wall of the urethra on a gradual slant in the younger fetuses but in older ones their rise through the prostate is a very sudden one until they are within a short distance from the lumen of the urethra, then they run along parallel to its axis for a considerable distance so that their course in the prostate is very much like that shown in fig. 10 in all of the older specimens. They open on the sides of the verum montanum in such a way that there is a small area of tissue over them which, if the slightest amount of pressure were exerted upon it, would close them most effectively. In fact their whole course in the verum montanum parallel with the axis of the urethra near its lumen makes for a closure of the duct in case of distention of the urethra.

6. The urticulus prostaticus in the thirteen weeks old fetus is observed as a very small lumen between the vasa deferentia. It descends between them, and just below the beginning of the

ejaculatory ducts becomes much larger than either of these structures. It passes through the wall of the prostatic urethra in company with them and opens in the midline just below the openings of the ejaculatory ducts. In the sixteen weeks old fetus the utricle seems to be composed of two partially fused tubes. Its upper end is obliterated and nothing is seen of it until the point is reached where the ejaculatory ducts and their envelopes are entirely within the posterior wall of the prostatic urethra. It opens below the mouths of the ejaculatory ducts. The utricle begins between the ejaculatory ducts before they enter the prostate in the twenty-two weeks old fetus as shown in fig. 6. It is very much larger than those observed in other specimens and shows other peculiarities already described. In all of the specimens older than twenty-two weeks the utricle appears only in the tip of the verum montanum. It opens in the middle line and in nearly every case below the openings of the ejaculatory ducts. In none of the specimens studied has there been found a single case in which an ejaculatory duct for a prostatic tubule opened into the utriculus prostaticus. There is no evidence that its mouth is protected in any way from invasion of substances or organisms in the posterior urethra.

7. Below the apex of the prostate there is noted in some cases a large number of glands of Littré. They are quite large and some of them have branches. They do not extend into the muscular layers of the urethra. They become very few in number lower down in the urethra.

CONCLUSIONS

1. The mucosa is always free from folds over the fetal trigonum vesicae. The musculature of the bladder wall, trigonum, and sphincter begins to develop at the thirteenth week and by the sixteenth week is very pronounced.

2. The subtrigonal glands begin to develop at the twentieth week. They are found at all ages after that, are few in number and insignificant in appearance, their only importance being that they occupy a strategic position where a small pedunculated enlargement might cause great obstruction to urinary outflow.

3. The subcervical glands of Albarran are constant after the age of sixteen weeks. They are similar in structure but are more numerous and larger than the subtrigonal tubules. Their importance also lies in their position. They originate from the floor of the urethra within and below the internal sphincter and grow back under the mucosa so that they lie directly within it. Hence a slight increase in their size would cause very grave obstruction to urinary outflow.

4. The prostate gland originates from five independent groups of tubules which begin to develop at about the twelfth week as follows:

(a) *The middle lobe* is made up of nine or ten large branching tubules originating on the floor of the urethra between the bladder and the openings of the ejaculatory ducts. There may be an absence of the middle lobe in which case there may be an ingrowth of tubules from the lateral lobes to form a commissure beneath the urethra. Embryologically the middle lobe is an independent structure. Practically it makes no difference because it is not separated by a capsule from the lateral lobes. The middle lobe is rarely absent, being found definitely present in ninety-seven specimens examined, possibly lacking in five others and definitely absent in one.

(b) *The right and left lateral lobe* tubules originate in the prostatic furrows and from the lateral walls of the urethra. They are composed of from twenty-seven to forty-six tubules which grow back to form the main part of the base of the prostate. They are well separated from each other by the anterior lobe and commissure, the urethra, the middle lobe and the ejaculatory ducts. Posteriorly they are separated from the posterior lobe by a fibrous capsule.

(c) *The posterior lobe* is an independent structure being made up of tubules which originate from the floor of the prostatic urethra below the openings of the ejaculatory ducts. They grow back behind the latter structures and are in no sense a glandular commissure as they are definitely separated from the other parts of the gland. The posterior lobe is the part of the prostate palpated per rectum and is an important consideration in the per-

formance of perineal prostatectomy. Hypertrophy rarely or never occurs in it and primary carcinoma of the prostate rarely or never begins anywhere else (Boyd and Geraghty).

(d) *The anterior lobe* is fairly large until the sixteenth week after which time it becomes greatly decreased in size and in the number of its tubules. It was found in all of the microscopic specimens studied but shrinks into insignificance at the twenty-second week. There is evidence in the occasional finding of enlarged anterior lobes at autopsy that this structure may persist throughout life.²⁴

The number of openings of prostatic tubules into the urethra is usually said to be between twenty and thirty. My studies have convinced me that this number is too low as in the specimens here recorded the number of tubules opening into the urethra varies from fifty-three to seventy-four, the average being sixty-three.

5. The vasa deferentia in early fetal life are comparatively speaking very large, being greater in size than the urethra at the thirteenth week. Their lumina broaden out behind the vesical sphincter at this time showing the earliest appearance of the ampullae. They become relatively decreased in size after the sixteenth week.

6. The seminal vesicles begin to develop as lateral evaginations from the vasa deferentia at the thirteenth week. They grow backward and laterally becoming more or less tortuous and send off as many as four short tortuous branches. In the later stages they communicate through a very narrow duct with the vasa deferentia just within the base of the prostate gland.

7. In the younger fetuses the ejaculatory ducts pass obliquely through the posterior wall of the prostatic urethra forming with their envelopes the verum montanum. In the older specimens their course through the prostate is not so regular. At the base they progress on a gradual slant until the middle of the gland is reached, where they rise quite sharply until they lie in the top of the verum montanum, after which their course lies parallel to

²⁴ Kuznitzky found a persistent ventral lobe in one out of every fifteen prostates.

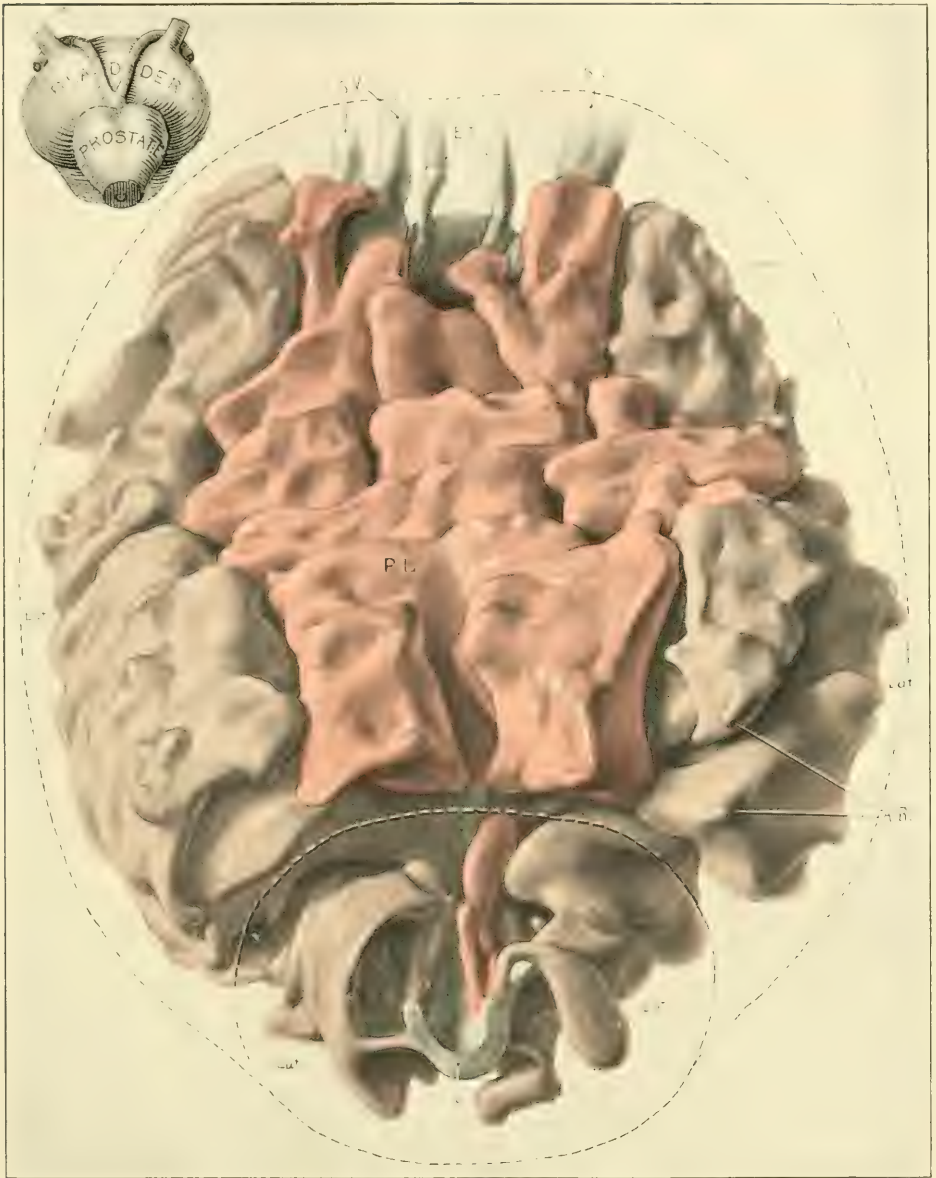
the axis of the urethra for some distance. They open into the urethra on the sides of the verum montanum, their mouths being composed of a collapsible fold of tissue so that pressure within the prostatic urethra very effectively closes them.

8. The fused Müllerian ducts may persist intact until the thirteenth week, after which time the lower end (utriculus prostaticus) which has become quite large in size and surrounded by a rather dense layer of stroma cells, contracts until after the twenty-second week when it is found only in the tip of the verum montanum and is relatively very small in size. It usually opens in the midline just below the ejaculatory duct openings and rarely if ever is there an ejaculatory duct or a prostatic tubule opening into it.

Description of a wax model of prostate of a new-born infant

The sections from which this model was constructed were cut 30 micromillimeters in thickness. Every fifth section was magnified twenty times and drawn by means of the projection apparatus in use at the Anatomical Laboratory of the Johns Hopkins University. These drawings were traced upon wax plates 3 mm. in thickness, the tubules and their branches first being identified microscopically with great accuracy. The wax plates were then cut in such a way that the bladder lumen and those of the prostatic tubules were left with bridges of wax between to preserve the exact contour. The wax plates were then piled, the axis of the bladder and urethral lumen and the lumen of the ejaculatory ducts being used as points upon which to build. The prostatic tubules are represented with their branches grouped, it being obviously impossible to represent every branch of each tubule. The various parts of the model are held together by means of pins and copper wire so that the exact position of the various structures represented is maintained. The various parts of the model are painted with several coats of different colored enamel to make clearer the different structures reproduced.

In conclusion I wish to express my thanks to Drs. Clark, Mall and Young for many suggestions which were of great value to me in this investigation.



The diagram in the corner of each plate shows the exact region of the prostate reproduced in the drawing.

Fig. 9 Dorsal view of a wax model of the prostate of a new-born infant. $\times 14$. *Lat.*, lateral lobes; *P.L.*, posterior lobe; *E.J.*, ampullae of vasa deferentia; *S.V.*, seminal vesicles; *A.B.*, anterior branches of lateral and posterior lobes; *U.*, urethra.



Fig. 10 Sagittal view of a wax model of the prostate of a new-born infant. $\times 14$. *Lat.*, anterior branches of lateral lobes; *P.L.*, posterior lobe; *E.J.*, ejaculatory duct; *S.V.*, seminal vesicle; *A.L.*, anterior lobe tubule; *U.*, urethra; *U.P.*, utriculus prostaticus; *A.G.*, subcervical glands of Albarran; *M.L.*, middle lobe tubules; *L.Ur.*, left ureter; *Bl.*, bladder; *P.Gl.*, prostate gland.

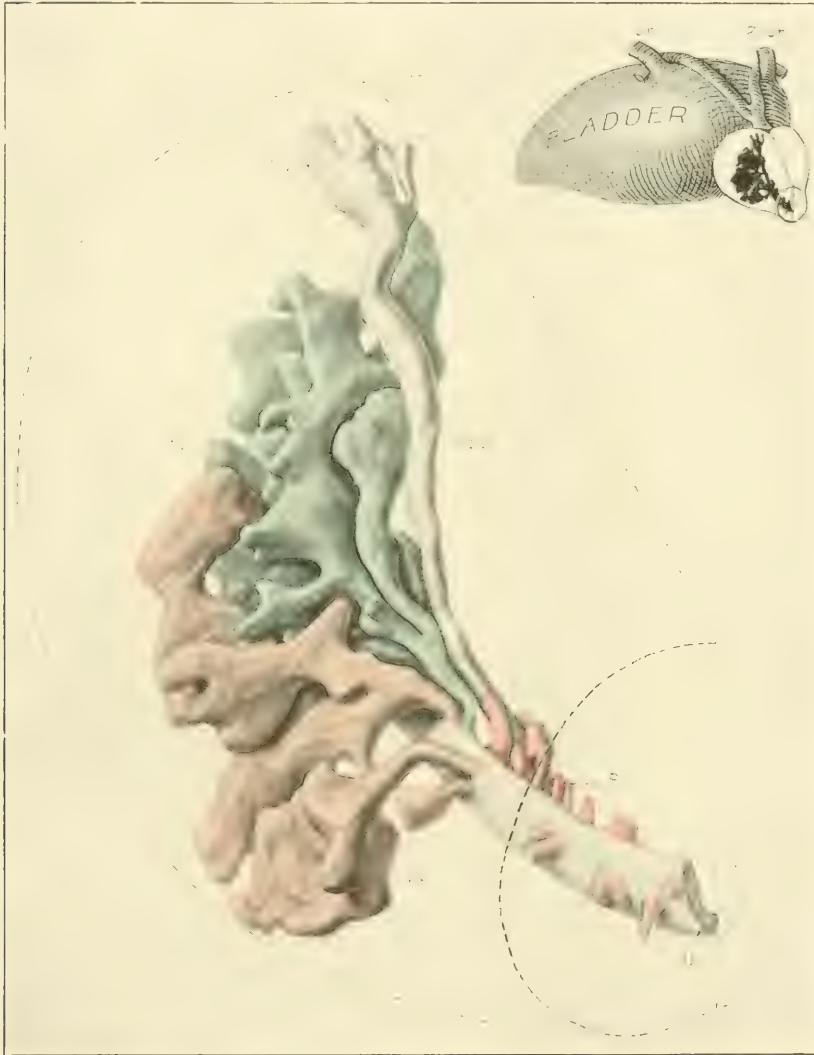


Fig. 11 View of wax model of the prostate of a new-born infant with posterior and most of lateral lobe removed. $\times 14$. *Lat.*, lateral lobe; *E.D.*, ejaculatory duct; *S.V.*, seminal vesicle; *U.*, urethra; *M.L.*, middle lobe tubule; *P.L.*, cut ducts of posterior lobe; *L.Ur.*, left ureter; *R.Ur.*, right ureter.

FURTHER OBSERVATIONS ON LIVING GROWING LYMPHATICS: THEIR RELATION TO THE MESENCHYME CELLS

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EIGHTEEN FIGURES


In an earlier paper,¹ read before the American Association of Anatomists in December 1908, the results were given of a series of observations on the method of growth of lymphatic capillaries as revealed by a study of the transparent fin expansion of the tail of living frog larvae. The present paper of which the essential parts were presented to the American Association of Anatomists in December 1910, represents the results of a newer set of studies, made during the spring of 1910.

The methods employed in the second study were identical with those used in the first; they deserve a fuller description than has been given. As there stated, two factors are essential to the success of the observations, an upright chamber and chlore-tone anesthesia. The former allows the larva to remain in its normal upright position while being watched; the latter keeps it motionless, without seriously interfering with the circulation of the blood or with the growth of the tissues.

The upright chamber which was used is by no means new to the histological laboratory. The earliest description of such an apparatus which I have found is by Cori.² In the article in *Zeitschrift für wissenschaftliche Mikroskopie* he describes the first apparatus, which he devised, as follows:

¹ E. R. Clark, Observations on living, growing lymphatics in the tail of the frog larva. *Anatomical Record*, vol. 3, no. 4, 1909.

² Cori, *Lotos*, Bd. 13, referred to in *Zeitschrift für wissenschaftliche Mikroskopie*, Bd. 10, 1893, p. 149.

Nach der in der Zeitschrift *Lotos* gegebene Beschreibung bestand aus einem Objectträger von Format 5:10 cm., auf welchem ein  formig gebogener Glasstreifen aufge kittet war. Derselbe functionirte als Seitenwände und Boden des Aquariumraumes. Die Rückwand dagegen bestand aus einem Deckglaschen vom Formate 30:40 mm. Die ganze Vorrichtung wurde mittels Klammern wie ein Objectträger auf dem Tisch eines umgelegten Mikroskopes befestigt.

An upright chamber with the tube of the microscope horizontal was used by Professor Mall in unpublished studies on the

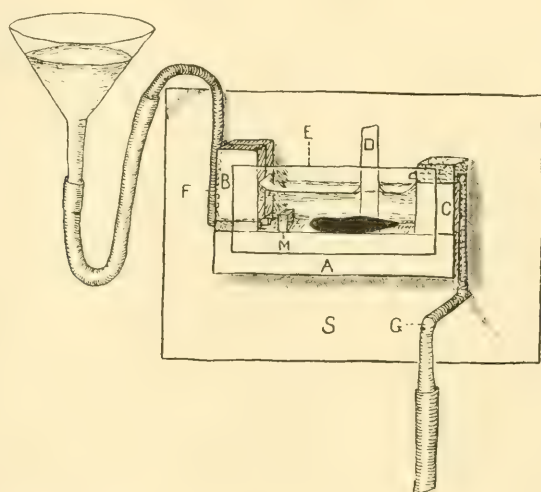



Fig. A Apparatus used in making the observations; for explanation of the letters see text.

blood vessels in the tail of the frog larva. Harrison³ used a 'glass cell having plane walls' to hold living frog larvae in their normal position while being photographed. Since the upright chamber is quite important for the success of the observations, a description of the one used will be given. It corresponds almost exactly with the one described by Cori. On an ordinary 51:76 mm. glass slide fig. A (S), are fastened, with the aid of damar or Canada balsam, three narrow strips consisting of two thicknesses of window glass, in the position  The uprights, (B) and (C),

³ R. G. Harrison, *Archiv für Entwicklungsmechanik der Organismen*, Bd. 7, 1898, p. 434.

are fastened to the horizontals (*A*) by damar. The fifth side of of the chamber is formed by a cover-slip (*E*), while the sixth side is left open. Paraffin is used in order that repair may be quickly made if the cover-slip is cracked or broken. A paraffin coating of the floor of the chamber was found to be useful. Such a cell has a thickness of about 5 mm., and a depth of from 10 to 12 mm. For larvae of more than 20 mm. in length, a thicker cell is necessary.

In use the slide is clamped to the stage of the microscope which is arranged with the tube horizontal. The tadpole to be examined is anesthetized in a small dish of chloretone solution, and is transferred carefully from dish to cell by means of a pipette or medicine dropper. The tadpole is brought carefully to the side of the cell next the cover-glass by means of a blunt needle, or by washing with a pipette. A narrow piece of cover-glass (*D*) is set on the floor of the cell, leaning against the cover-glass (*E*) which forms the front wall of the cell, and is brought against the portion of the tail to be observed, by pressing gently with a needle. In this way the part of the tail to be studied is held firmly in contact with the cover-glass, and is protected from the jarring of the water. Since the narrow piece of cover-glass comes in contact with the thick central muscular portion of the tail, pressure on the fin expansion is avoided. For older larvae, which require more frequent changes of the chloretone solution, a special apparatus was designed, by which a continuous circulation of chloretone through the cell may be maintained. It is this modification which is shown in fig. *A*. The solution is poured into a funnel, which may be raised or lowered as desired. A rubber tube conducts the fluid to a fine glass canule (*F*) which enters the chamber at the lower corner. A small block of paraffin (*M*) protects the tadpole from the direct stream. A second glass canule (*G*) carries off the excess of fluid from the opposite upper corner. It is possible, with a cell so constructed to make observations with the highest powers of the microscope, even with the oil immersion, for a cover-slip, only, separates the microscope objective from the object studied.

For making records the camera lucida (E. Leitz drawing eye-piece no. 112), with which the plane of the drawing-board makes an angle of forty-five degrees with the tube of the microscope, was used. A board, set at this angle to the horizontal, is placed directly below the microscope, and is held firmly between clamps. The outline sketches, made with the aid of the camera lucida were usually corrected with a higher power ocular.

Chloretone (acetone-chloroform), the anaesthetic properties of which were discovered by Abel, and which is rapidly replacing other substances as an anesthetic for small organisms, because of its efficiency and harmlessness, is a well-nigh perfect anesthetic for tadpoles. At the stages on which these studies were made it inhibits bodily movements and respiratory movements; while the movements of the alimentary canal are unaffected, and, if the proper strength is found, the force of the heart beat remains undiminished. The strength of chloretone necessary to produce the proper depth of anesthesia varies somewhat with the species of tadpole. Even for the same species, individual differences are met with, so that a set rule cannot be laid down. In general, a solution made by dissolving 1 gram of chloretone in 5,000 parts of tap water suffices. With larvae of *hyla pickeringii*, 1:3,000 may be used safely. For larvae of *rana sylvatica*, *r. palustris*, and *r. catesbiana*, the necessary strength varies between 1:4,500 and 1:6,000. It was found most convenient to make up a series of dilutions containing 1:3,000 to 1:6,000 parts of chloretone. With these on hand the strength may be varied according to indications. Since chloretone is somewhat volatile, fresh chloretone must be added from time to time during the observations. The 'indications' which controlled the strength of chloretone to be used, were, on the one hand, the return of muscular movement, on the other, the weakening of the heart action. Evidence of the latter is to be seen in the condition of circulation in the blood vessels of the tail, which are in view throughout the observations.

The anesthetization of *hyla pickeringii* larvae is much easier than that of the other three species mentioned. With a solution of between 1:3,000 and 1:4,000 one of these larvae has been kept under anesthesia five to twenty hours a day for more than four

weeks without seriously interfering with heart action or the growth of the tissues. The anesthetization of the other species offers many difficulties. Often from a half-hour to an hour or even more may be taken up in the attempt to bring the animal to the proper point of anesthesia, where muscular movements are lost and the heart beat is vigorous. Occasionally the heart beat stops entirely. In such a case it will usually be resumed as the result of a delicate massage of the heart produced by rhythmically forcing a stream of water against the heart region with a medicine dropper. The difficulties of anesthesia increase as the larva grows older. It was thought that this might be due in part, at least, to the greater need for oxygen. Whether this explanation is true or not, certain it is that frequent changing of the chloretone solution greatly assisted the anesthesia. This is most conveniently done by employing the modified apparatus described, with which a continuous circulation of the chloretone solution may be kept up. During the intervals between observations the larva is returned to fresh water.

A short review of the results of the observations previously reported will now be given. It was possible to see clearly the individual structures present in the fin expansion of the tail. Lymphatics, blood vessels, nerves, mesenchyme cells with their branched processes, red and white blood cells, stand out with remarkable clearness. The immobility of the object, which may be maintained for hours, makes possible both prolonged observations of a small selected area, as well as careful records of small or large areas. It is possible to watch, from minute to minute, the changes which go on in a selected cell or process, and it is also possible to make accurate drawings of the entire blood-vascular or lymphatic plexus of both fin expansions.

Among the results of this first series of observations, in which the structures were studied both extensively and intensively, it was found that lymphatics grow by a process of sprouting, in which, so far as concerns the material of which their endothelium is formed, they maintain a complete independence of blood vessels, mesenchyme cells, and wandering cells. In the species studied the blood vessels precede the lymphatics, in their invasion

of the fin expansion. The lymphatics grow in as outgrowths from the dorsal and ventral longitudinal lymph trunks, and soon catch up to the blood capillaries; from then the two systems are practically coextensive. Careful studies were made of the two sets of vessels, as they developed side by side, and each was found to be quite independent of the other. Blood capillaries were watched through their various transformations. Some were seen to be converted into arterioles or venules as the capillary area increased, while others were watched through the stages of atrophy, solidification and retraction. Neither new nor atrophying blood capillaries contributed tissue to the lymphatics.

The lymphatics are apparently quite uninfluenced by the blood capillaries. They have a specific independent life of their own. If a capillary is selected and carefully observed, it is found to be in a state of perpetual activity. Fine pointed protoplasmic processes are being continuously sent out at the sides and tip. These processes reach varying lengths and most of them are again withdrawn. The lumen of the capillary may, however, extend into the base of one of these fine processes, and, gradually extending, lead to an increase in the length of the capillary, or to the formation of a branch. A branch which is at first without a nuclear area, will, if it continues to grow, receive one from the parent stem. That these growth processes are intimately associated with functional activity was suggested by the observation that extravasated red blood cells may be taken into the interior of the capillary, through one of these finely pointed processes.⁴

⁴ A. Dziurzynski (Untersuchungen über die Regeneration der Blut- und Lymphgefäße im Schwanz von Froschlarven, in *Extrait du Bulletin des Sciences de Cracovie*, serie B: sciences naturelles, March 1911) states, in a footnote, pp. 207-208, that he has failed to observe the taking up of red blood cells by the lymphatic capillaries, although he sought eagerly for it. The accuracy of my observation of this unexpected process is hardly open to doubt. It was seen many times and with perfect clearness, and has been verified in the studies which are to be reported in the present paper, on *Hyla pickeringii* larvae. The illustrations of the various stages, given in my earlier paper, (E. R. Clark, *Anatomical Record*, vol. II, no. 4, 1909, figs. 6 and 7) are not exaggerated. It must be concluded, then, that for some reason Dziurzynski has missed seeing the process. I am inclined to believe that his failure is due to the crudity of his method of observation. On page 189 he states that his larvae were placed on a slide, with a cover-glass over the tail. Also that he was able to observe a larva for only one, or, at most,

A few observations were made on the relation between the growing lymphatic and the mesenchyme cell. Here again, as in the case of the blood capillaries, while this point was not subjected to such rigid tests, nothing was seen which would suggest that there is any transfer of tissue from mesenchyme to lymphatic.

The finding of larvae of *hyla pickeringii*, during the spring of 1910, made possible a new set of studies. The tadpoles of this species offer peculiar advantages over those of *rana sylvatica*, *r. palustris*, and *r. catesbiana* which were previously used. They are smaller and more transparent, are free, for a longer period, from troublesome pigment cells, and are very much easier to anesthetize. But of much more importance was the discovery that, in the posterior portion of the tail, the lymphatics grow into the fin expansion of the tail in advance of the blood vessels. In fact, specimens may be met with in which, over a region corresponding to the posterior twenty muscle segments, the lymphatics have grown out in abundance, reaching almost to the fin margin, without a single blood capillary appearing beyond the edge of the central muscle mass (fig. 1).

The importance of this discovery will be appreciated readily. If, on further investigation, it be found that, in this area, no blood vessel has at any time been present, during the growing out of the lymphatics, the question as to the possibility of the growth of lymphatics independent of blood vessels will be settled. Obviously the only way to be certain is to select a portion of the lymph trunk before the branch has appeared, to watch its growth and see whether there is even a transitory appearance of a blood capillary near it which might contribute to its growth. If it be found that the lymphatics do indeed precede the blood vessels,

two hours, because of the return of movement. Had I used such a method I feel quite certain that I should also have failed to observe this process, for it was only by the use of the upright chamber, and by the most careful use of the chloretone so that the same larva could be observed for many hours at a time, and over periods of several days, that I made the discovery. Another possible explanation of the divergence of our results may lie in the difference in age of larvae studied. While Dziurzynski does not state the age of the ones used for this particular study, it may be inferred from his paper that he studied much later stages than I. The larvae which I studied were *rana catesbiana* larvae 8 to 9 mm. long.

such a region offers an unparalleled opportunity to study the relation between the growing lymphatic and the growing mesenchyme cell.

It seemed that both these points could well be taken up in one set of observations, for it is easily possible, while making a study of the growth of a lymphatic sprout from its very beginning, to note the presence or absence of a blood capillary in the neighborhood.

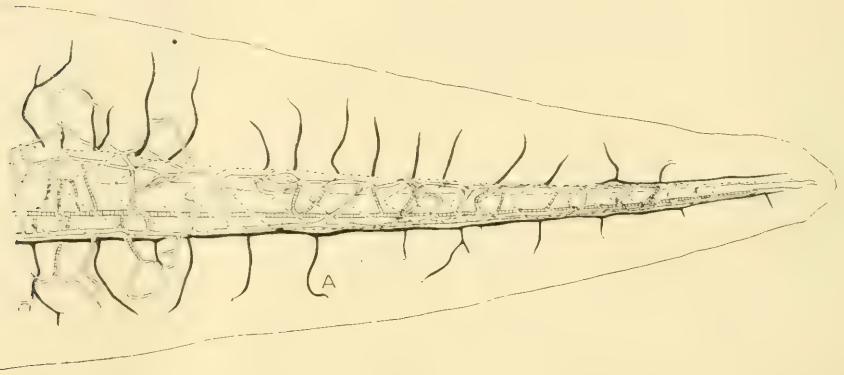


Fig. 1 Posterior half of tail of *hyla pickeringi* larva, showing region in which the lymphatics precede the blood vessels. This larva is the one on which the studies shown in figs. 2 to 14 were made. This drawing was made from the living larva on June 3, at the close of the observations. Lymphatics are in solid black, blood vessels in lines. The ventral caudal lymph trunk and a short stretch of the dorsal caudal lymph trunk are shown. A, the lymphatic sprout shown in figs. 2 to 14. The larva is shown in this figure in its normal position. In the other drawings the structures are shown in their reverse position, as seen under the microscope. Enlargement 25 times.

It is most convenient to anticipate results at this point enough to say that during the series of studies to be recorded, no blood vessel entered the region under observation.

In order to discover the exact relationship which the lymphatic endothelium and the mesenchyme cell bear to one another during their growth, it is first necessary to find out just how each one develops by itself, and then to study the relations between them when they develop side by side. For the mesenchyme cell, a separate study of the observable growth changes is easily possible,

since areas may be selected in which no lymphatic or blood vessel has appeared.

The exact method chosen for making the desired studies was as follows. In the tail of the tadpole the lymphatics first appear as two longitudinal vessels, which have been many times described, the dorsal and ventral caudal lymph trunks. In *hyla pickeringii* the dorsal trunk, save for a short distance at the tip of the tail, is concealed between the muscle layers. The ventral caudal lymph trunk, on the other hand, may course for considerable stretches, even for its entire extent out from under the muscle, so that it may be clearly seen. The caudal vein is situated just dorsal to the lymph trunk, while, further dorsally, ventral to the notochord, runs the caudal artery. The vein may be seen quite readily, especially toward the tip of the tail, where the muscle layer is thinnest. A stage may be selected in which neither blood nor lymph vessel has grown from the main trunks into the ventral fin.

It was planned to select for study a portion of the ventral caudal lymph trunk, at this early stage, before any branches had been sent out, to make a careful record of each individual mesenchyme cell around it, as well as of each mesenchyme cell in the entire region in which a sprout of the lymphatic would be expected to grow, and, in case a sprout were sent out in the region hoped, to make numerous successive studies and records of each individual mesenchyme cell, as well as of the lymphatic, as they developed side by side.

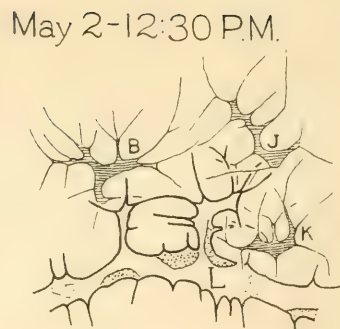
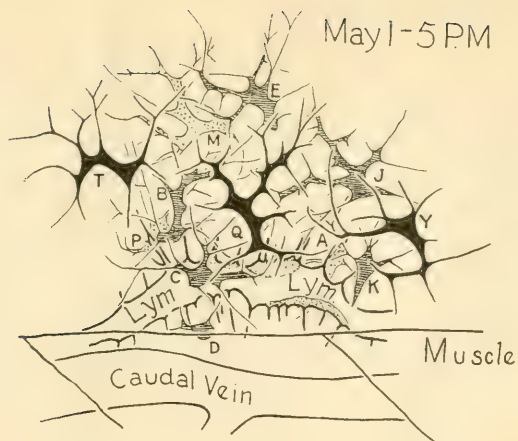
My hopes were amply fulfilled. In the selection of a portion of the lymph trunk, a place was chosen where a slight ventral bend occurred, as shown in fig. 2. The larva, at this time, was about 5.5 mm. long. At this spot the lymphatic was well away from the muscle edge, so that both walls of the lymphatic were clearly visible. It was also well separated from the caudal vein. The vein was easily recognizable through the very thin muscle layer. A drawing was made of the lymphatic, vein, muscle edge, and of the neighboring mesenchyme cells, with their main processes. The tadpole was then placed in fresh water over night. The following morning a lymphatic sprout had started to grow

out from the trunk at exactly the spot chosen. A new drawing was made of the various structures (fig. 3). The larva was kept under anesthesia the entire day, and frequent drawings made, figs. 3, 4, 5, 6, 7 and 8, for the lymphatic was in a state of very rapid growth. A record was made of the mesenchyme cells well in advance of the growing lymphatic, toward the edge of the fin. The following day a new record was made (fig. 9,) in which the area of recorded mesenchyme cells was extended to the fin margin. From this time on, for thirty-one days, records were made almost daily, and in each record there was included lymphatic, caudal vein, and all the mesenchyme cells, with their main processes, in the region selected. It is unnecessary to reproduce all the drawings. They were demonstrated at the meeting mentioned. Two later stages are selected (figs. 10 and 11) which were drawn at eight and at twenty days later than fig. 9. In addition to this series, numerous other studies were made on other regions in this same tadpole, as well as on other larvae. Fig. 15, for instance, in which are given the results of a study primarily of the changes in the capillary wall, is taken from the dorsal fin of another larva. The camera lucida drawings were made at an enlargement of 450x, and corrections were made at a still higher enlargement.

In order to simplify the description, the two tissues—mesenchyme and lymphatic—will be taken up separately. The relations between the two as they grow together will then be indicated.

The mesenchyme cells at the time at which they first become visible in the fin expansion of the larva of *Hyla pickeringii*, consist of an irregularly shaped thick central portion containing a nucleus which is not clearly visible during life. From this central portion extend a varying number of branched processes of different lengths, and of a thickness which diminishes from center to periphery. The branches in turn give off large numbers of minute fibrillae, which form a richly anastomosing network extending from epidermis to epidermis. These minute fibrillae may be seen and followed in the living larva, by careful focussing. They usually appear as minute dots which seem to travel up or down as the microscope is focussed. When any of these tiny

Figs. 2 to 11 Successive drawings of the same region (A in fig. 1) in the fin expansion of the tail of *hyla pickeringii* larva, to show the relation between the growing lymphatics and the growing mesenchyme cells. Drawings were made while the larva was anesthetized with chloretone. The individual mesenchyme cells, A to Z and 2 to 20, are represented by three forms; solid black: cells near the surface toward the observer; cross-lined: cells in interior; dotted: cells near the surface away from the observer. The same letters in the different drawings indicate the same cells. Where a cell has divided the two daughter cells are indicated as B^1 and B^2 . Between figs. 9 and 10, also between figs. 10 and 11, records were made nearly every day, which have not been reproduced, which show the intermediate positions of the cells and of the lymphatic. Lymphatic wall, Lym LL, is represented as somewhat thicker than it actually is. The nuclear areas in the lymphatic are represented by dots. In figs. 4, 5 and 6 the three cells shown B, J and K, are the ones in approximately the same layer as the lymphatic. Fig. 8 is included because it shows well the wandering into the sprout of a nuclear area (nucl), from the main stem. In figs. 4 to 7 this nuclear area is seen just starting into the sprout. Enlargement 300 times.



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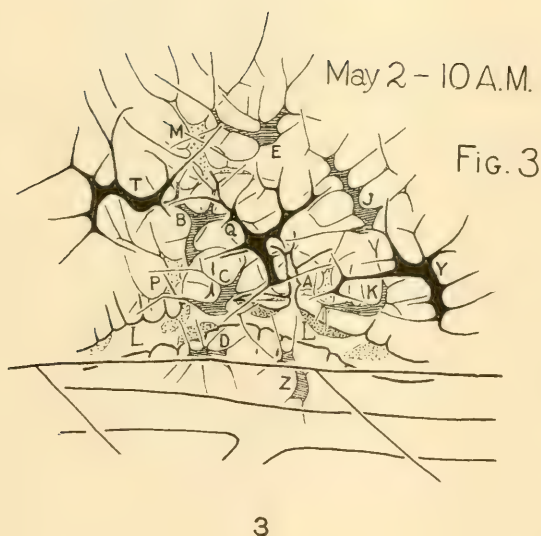
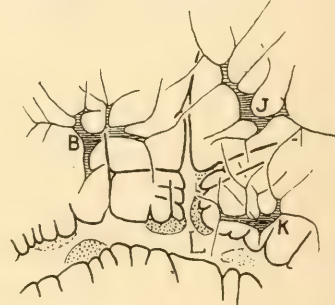
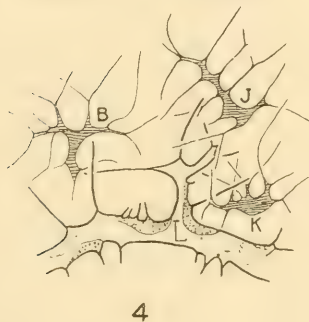


Fig. 3

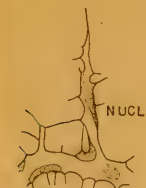
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May 11

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May 23

11

fibrillae is carefully followed, it is seen to lead to one of the larger easily visible processes. The behavior of these minutest fibrillae has not been considered in the present study—the drawings including only the processes which are readily seen. Between the larger processes communications are rare, though here and there undoubted connections may be seen. The branched mesenchyme cells have a uniform distribution, an equal amount of space separating the neighboring cells.

Near the edge of the muscle, where the distance between the two layers is greatest, the cells are arranged in three or four layers. Near the fin margin, where the fin is so thin that the two layers of skin are almost in contact with each other, the cells form but a single layer. Between these two extremes there are all gradations. In making records of the cells a color scheme was used to designate roughly the cells in the different layers. Thus, those nearest the observer were drawn in black, those farthest away in green, and those in the middle in red. In the reproductions, the form has been changed, as indicated in the legend of figs. 2 to 11. With this crude scheme, it was found to be very easy to identify each individual cell, with its main processes, in the successive stages.

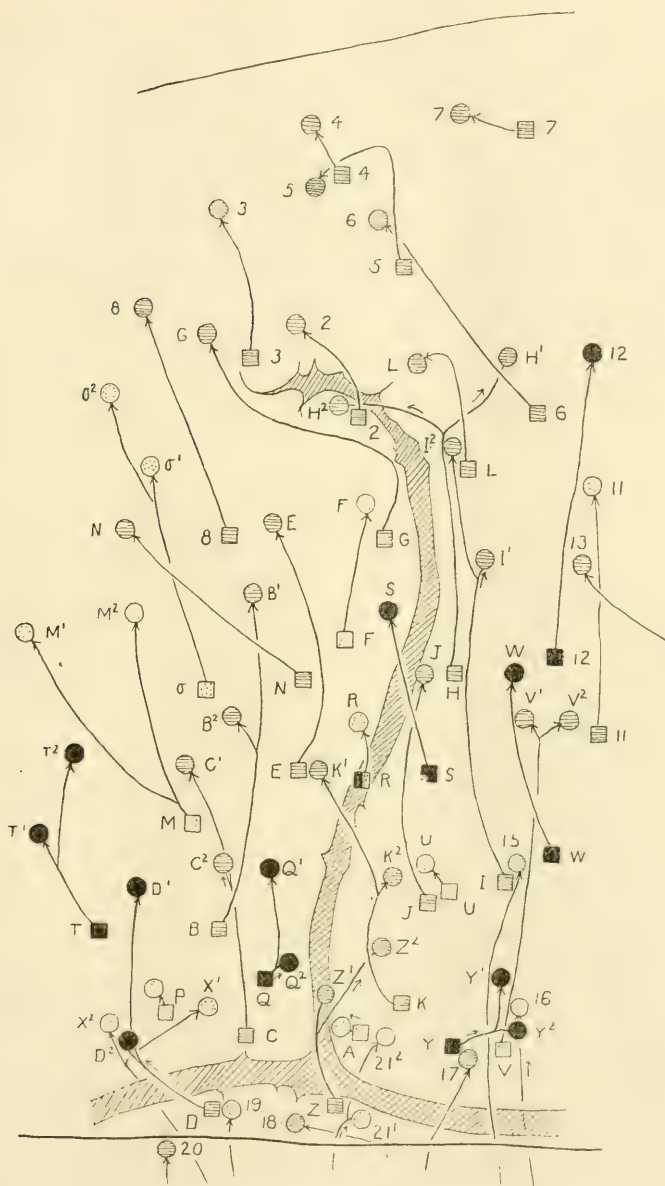
What now, are the visible changes which occur in these cells during the growth of the tail?

As successive stages are compared it is soon to be noted that changes are continually going on. On the one hand, branches become thicker and longer, and new branches are sent out. On the other hand, branches become shorter and thinner, until they may entirely disappear. The thickened central portion, too, is continually changing shape, now extending further and further along one or more branches, while retreating from others. The picture becomes much clearer when it is found that, by a summation of these processes, the cell may actually shift its position, or wander. The evidence for this conclusion does not rest upon the observation of a single cell or a group of two or three cells; nor does it depend upon the selection of some supposedly stable structure to serve as a fixed point—such as a chromatophore which may itself shift position. It is only certainly to be proved

by observing a considerable number of cells, by keeping records of the distance of these cells from relatively fixed spots and from one another, and by watching them over periods of time long enough so that the shifting in position is beyond question. Moreover the shifting must be such as cannot possibly be accounted for by the general growth expansion, of the entire organ, or by a mere mechanical dragging of the cell.

That there is a genuine wandering of the cell is shown by a study of the series of drawings from which figs. 2 to 11 are taken. The region selected was large enough, the number of cells watched was large enough, the length of time and number of successive observations were great enough and the changes in shape and position of cells were marked enough to exclude all other interpretations. A general idea of the relative amount of shifting is given in fig. 12, which shows diagrammatically the amount and direction of change in position of cells between fig. 9 and fig. 11 (May 3 and May 23). Here we see that, while certain cells such as *A*, *P*, *U* have remained in practically the same spot, others such as *B*, *C*, *I* or their daughter cells *B*^{1 and 2}, *C*^{1 and 2}, *I*^{1 and 2} have made long excursions while still others, such as *F*, *S* have made short excursions. While the difference in location of such a cell as *B* (*B*¹), with reference to the muscle edge may be in part accounted for by the general expansion of the entire fin, measurements show that the change is very much greater than could be accounted for in such a way. In fig. 9 (May 3) the width of the fin from muscle edge is 0.38 mm. The

Fig. 12 Diagram to show the amount and direction of change in position of the mesenchyme cells shown in the figs. 2 to 11. The same form for the cells is used—solid black, dots, and cross-lines to indicate the different layers. The squares represent the position of the cells on May 3 (fig. 9), the circles their position twenty days later, on May 23 (fig. 11). Since the fin increased in size during this time, the positions of the cells in fig. 3 were measured with reference to the muscle edge as a horizontal and a vertical passing through the base of the lymphatic sprout, and were referred to a similar horizontal and vertical in the diagram of the later stage, with increases to correspond to the growth of the tail. Where mitotic division has taken place, the line, which shows the change in position, forks. The arrows represent the direction of wandering. In cases where cells came into the field, which was being watched, after May 3, the square is omitted (cf. cells 13, 15, 16, etc.)



distance of cell *B* from muscle edge is 0.078 mm., or 29.5 per cent of the entire width. On May 26, seventeen days later, three days after the stage shown in fig. 11, cell *B*¹, one of the daughter cells of cell *B* is 0.29 mm. distant from the muscle edge, or 58 per cent of the entire width, 0.50 mm. Thus this cell has moved through 37.5 per cent of the width of the fin, an actual distance of 0.188 mm. Calculated in a similar way, the cell *P* is found on May 3, 13.5 and on May 26, 14 per cent of the distance from muscle edge to fin margin; it has, then, remained practically stationary. Many striking instances of relative change in position may be seen by following any two or three selected cells through figs. 7 to 11, such as cells *E*, *N* and *F*.

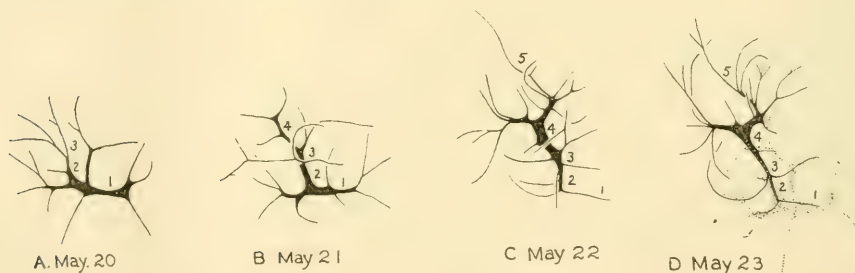


Fig. 13 Four successive drawings of the same mesenchyme cell, taken from the series of studies represented in figs. 2 to 12, to show the method of wandering of the mesenchyme cell. The cell here shown is the one labelled *G* in figs. 2 to 12; stage *D* in this figure corresponds to cell *G* in fig. 10. The dotted cell in *D* is the same as *A*. It is superimposed to show the change in position. Enlargement 270 times.

The exact way in which this change in position of the cells takes place is shown by a comparison of successive drawings of the same cell. Cell *B*, in figs. 2 to 9, furnishes a good example, since the several stages here are separated by short intervals of time. In fig. 13 a single cell is shown, in four successive stages at daily intervals. It will be seen that change in position is effected by a movement of the protoplasm. Fine processes are sent out from the main body of the cell, such as 3, in fig. 13. These processes may be quite temporary, and may be withdrawn subsequently, without attaining more than a hair-like thickness.

They may, however, grow thicker as new processes are sent out beyond. This increase in thickness takes place at the expense of other portions of the cell. Thus, while (fig. 13) processes 2, 3 and 4 successively increase in thickness, processes 1, 2 and 3 decrease. In fig. 13 *A*, the main body of the cell lies at 1, in fig. 13 *D*, process 1 is merely a short hair-like filament. The mode of progression, then, seems to be a true amoeboid one, since change in position is brought about by the sending out on the one side and the withdrawal on the other of cell processes, with a shifting of the main body of the cell from the retreating to the advancing processes. That the wandering is an active process and not the result of a purely mechanical pulling and pushing, is shown by the character of the changes which the cell undergoes. It is impossible that such changes as are shown in fig. 13 could be produced by a merely mechanical dragging and pushing.

The rate of progression of the individual cells varies. Wandering is most marked in the cells in the interior of the fin, while those near the surface are almost stationary. The former are represented with cross lines in the drawings, the latter are dotted or solid black. The cell *B* between fig. 2 and fig. 9, two days, wandered about 22 micra. Cell *G* shown in fig. 13, wandered in three days about 44 micra. Roughly speaking since the thicker portion of the cell measures from 25 to 30 micra in length, the main body of the cell may wander a distance equal to its own length in two to three days. It is probable that this is rather low for the normal maximal rate, since the prolonged use of chloretone causes a slowing of the growth processes. Moreover, during most of the time over which the observations extended, the weather was unusually cold, which also causes a retardation in growth. Since growth processes are much more rapid in the regenerating than in the normal tail, it is probable that a very much higher rate of wandering may be observed there.

The direction of wandering is principally toward the free margin of the fin, as may be seen by a glance at fig. 12. During the observations many cells, such as cells 15, 16, 17, moved out into the fin from the space between the muscle layers. A small amount of antero-posterior wandering occurred, as seen in cells *H*¹ and

H^2 , X^1 and N . A few cells such as cell D , (D^1 and D^2) moved from the interior toward the surface. One cell, R , which for a time was spread out in part under one epidermal layer, and in part under the other, gradually withdrew one set of processes until it was eventually entirely on one side.

In addition to the amoeboid wandering, another striking change takes place in these cells, namely, mitotic division. This process has been figured and briefly described by Haidenhain.⁵ My findings agree in the main, with his, the chief difference being that, while he states that the cells after division become fixed, I find that, while movements are most rapid at the time of division, yet after, as well as before, the cells are not 'fixed' but are distinctly motile. The changes which take place during the division of the cell are as follows (fig. 14). The thick central portion of the cell swells and the processes become shorter and thicker at the base and some are entirely withdrawn (fig. 14, A and B). The spireme appears as dark spots which soon form in a line across the equator of the cell (fig. 14, C), this line divides rapidly and the chromosomes as two sets of lines move in opposite directions to the poles, where they shorten and become moulded into spheres, the two daughter nuclei. These nuclei are clearly visible for only a short time. The moving apart of the chromosomes takes place rapidly—so rapidly in fact that the movement may be seen. Four minutes after their arrangement in the equatorial plane, they are well separated, and form the typical groupings at the poles. Five minutes later the rounded nuclei are formed and fifteen minutes later the nuclear outlines are lost and the nuclei can no longer be distinguished in the living cell. The remainder of the cell with all of its processes shortened and some entirely withdrawn has a clear, glassy appearance during division. The concentration of the protoplasm in the neighborhood of the nucleus is most marked at the time when the chromosomes are moving apart. As soon as the chromosomes are well separated, the cutting in of the protoplasm commences. The pear-shaped body of the cell lengthens to a cylindrical shape,

⁵ Haidenhain, *Plasma und zelle* 2e. Lief. pp. 721 and 722 in *Bardel Lebens Handbuch der Anatomie des Menschen*, Jena, 1911.

and, at the center, a ring-like depression occurs. This increases quickly in depth until, in three or four minutes, the two daughter cells are connected by only a narrow process (fig. 14, *E*). At this time the retraction of the processes is most pronounced, while the ones not entirely retracted are extremely thin. Soon however, what seems like a relaxation of the protoplasm occurs, and the two daughter cells expand and commence to send out

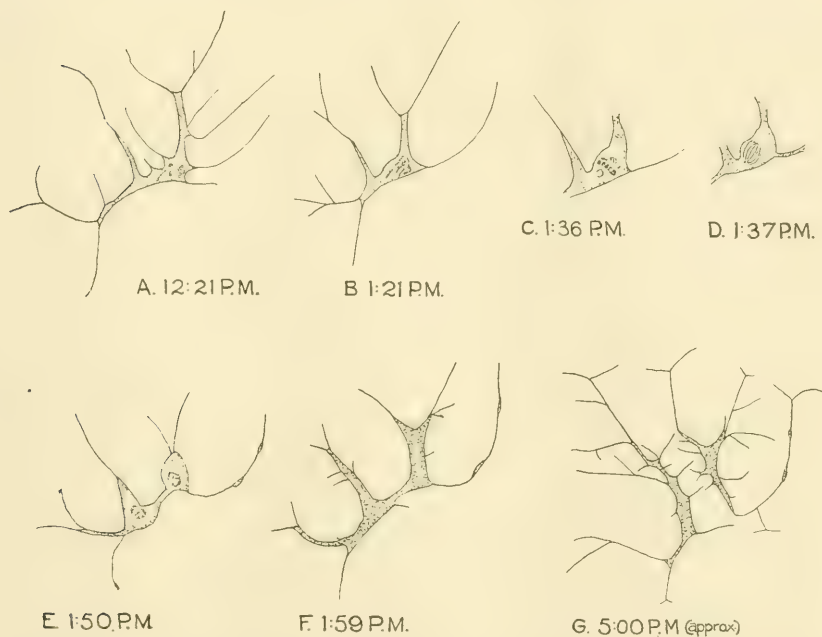


Fig. 14. Drawing to illustrate the mitotic division of a mesenchyme cell, from same larva as shown in figs. 2 to 13; *C* and *D* are incomplete. This division was observed on May 22. The position of the two daughter cells on the following day, May 23, is shown in fig. 11, *Z*¹ and *Z*².

processes. For some time a slight process connects the two cells, but after about two hours, they are completely separated. The sending out of new processes takes place rapidly. For a time after the disappearance of the nuclei the two new cells have a finely granular appearance, which distinguishes them from the other cells. This is gradually lost, and eventually the new cells take

on the character of the non-dividing cells (fig. 14 *G*) and slowly wander away from each other. Cells Z^1 and Z^2 , fig. 11, show these same two daughter cells on the day following.

The position of the cell in the fin at the time of division is of interest. A study of fig. 12, in which the position at the time of division is indicated by the point at which the line showing the course of the cells forks, shows that more divisions occur in the thicker portion, of the fin near the muscle than in the thin part near the edge. Here cells 2, 3, 4, 5, 6, 7, 8 and *L* remain undivided. Of the cells in the thicker portion, those in the interior appear more likely to divide than those near the surface—the cells *A*, *P*, *U*, *R*, *S* and *V* remain undivided. In all, nearly one-half of the cells observed underwent division during twenty-three days. None of the daughter cells arising from division, underwent further division during this period.

As a summary of the results of this series of observations on the growing mesenchyme cells the following may be said. All the mesenchyme cells in a selected strip of the fin expansion extending from muscle edge to fin margin were carefully watched and recorded from day to day over a period of twenty-three days. During this time the larva increased in length from about 5.5 mm. to 8.5 mm., while the width of the ventral fin expansion increased from .38 to .50 mm., at the portion selected. It was possible to identify each cell throughout this period, and to study its growth changes. The cells were found to possess two striking properties, those of amoeboid movement and of mitotic division. Every mesenchyme cell that was present in the area selected at the beginning of the observations, as well as every cell which wandered into this area from between the muscle layers or from the more anterior portion of the tail during the observations, maintained always its identity as a mesenchyme cell. Although the cells were actually watched for hours at a time, during periods of active growth changes, and though a number of mitotic divisions were watched throughout, there was never the slightest indication of the transformation of one of these mesenchyme cells into a cell of another type. The mesenchyme cell remained mesenchyme cell. The two daughter cells quickly resumed the typical

form and the characteristic power of amoeboid movement of the non-dividing mesenchyme cell. There was nothing whatever to indicate that either the non-dividing or the dividing mesenchyme cell may give off, by bodily transformation or by budding, a leucocyte or a lymphatic endothelial cell. The mesenchyme cell, then, during this period of growth, has a specific, independent life.

Let us now turn our attention to the growing lymphatic. To former observations, which have been confirmed in the present study, some new observations have been added which make the story more complete. The newer work has been concerned particularly with a more careful study of the nuclear areas.

The nuclear areas occur at somewhat irregular intervals along the wall. When seen in profile, they consist of a thick clear central portion, surrounded by a granular zone, which extends for a considerable distance longitudinally along the lymphatic in both directions—gradually growing thinner. The term nuclear area is used because the nucleus which may be clearly seen by fixation and staining, merges imperceptibly into the perinuclear area in the living larva (fig. 15). When seen en face the clear central portion is invisible, but the position of the nuclear area is clearly indicated by the granules surrounding the nucleus, so that it is possible to keep track of all the nuclear areas in a sprout.

If a study is made of the behavior of the nuclear areas it is found that there are two distinct processes going on, wandering and mitotic division. These will be taken up separately.

The newly formed sprout receives its first nuclear area from the parent stem by an in-wandering along the wall. As the branch grows, it may receive a second and a third nuclear area from the the main stem. How many may wander into a branch cannot be stated; I have watched as many as five pass into a branch, (cf. fig. 16, nuclei 4, 5, 6 and 7). Once in the branch they do not remain at rest, for they are continually moving up or down the lymphatic, or from side to side, along a spiral course (fig. 16). They have a tendency to arrange themselves in pairs on opposite sides of the lymphatic (fig. 16, nuclei 3 and 4). They may however remain single, or from groups of three or four. In one instance, in a sprout which was under observation, four nuclear

areas grouped themselves so closely together near the tip of the sprout that they could hardly be recognized individually, until they separated again. The nuclear areas do not maintain the same relative position in a branch, for one may actually move

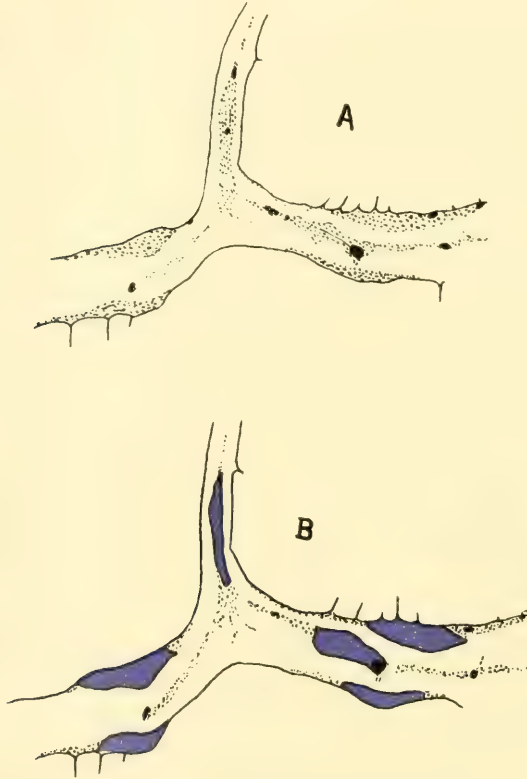


Fig. 15 Drawings of lymphatic in the tail of *hyla pickeringii* larva, to show the relation between the nucleus and the rest of the nuclear area. A was drawn while the embryo was in alcohol, unstained. B shows the same lymphatic, while the embryo was still in alcohol, after staining with hematoxylin. The large black dots represent pigment granules. Oil immersion.

past another (compare in fig.16 the relation between nuclei 2^b and 1^b in stages J, K and L).

The nuclear areas which have once passed into a branch, do not necessarily remain in that branch. They may pass into the

base of the branch for a short distance and then retreat, to continue their movement along the main stem. (fig. 16, nuclei 2^a and 2^b in stages *F* to *J*). Moreover, often in the developing lymph system, a branch which is quite active at one time, is apparently found to be superfluous later. It then becomes narrower and shorter and the nuclear areas, which may be present, retreat to the parent stem.

These nuclear movements furnish strong evidence for the view that the endothelium of the new lymph sprouts is a syncytium. That one nucleus could move past another and that such changes in relative position as are shown in fig. 16 could take place, if there were definite cell boundaries seems hardly conceivable. Many observers have tried in vain to find, by the use of silver nitrate, outlines of endothelial cells in the lymphatic sprouts in the tail of the frog larva. I have injected them directly with silver nitrate, but have failed to find endothelial markings. Moreover, the study of the wall of these capillaries in toto, in alcohol,⁶ as well as in sections stained with intense protoplasmic stains such as acid fuchsin, reveals a network of fibrillae which connect neighboring nuclear areas, thus forming a syncytium.

The other striking property possessed by the nuclear areas, which has been observed in the living larva, is that of mitotic division. Here, as in the case of the mesenchyme cells described above, the picture was unmistakable (fig. 17). The granules disappear, as the central portion becomes clear and spindle-shaped. As in the dividing mesenchyme cells spireme formation, arrangement of chromosomes, formation of daughter nuclei, and the cutting apart of the remainder of the cell are to be seen with surprising clearness. The chromosomes separate so rapidly that the actual movement may be seen. The cutting-in of the cell also takes place rapidly—from one to two minutes only being consumed. After division the two daughter nuclear areas move apart and maintain for a time a characteristic shape. Later

⁶ E. R. Clark, An examination of methods used in the study of the development of the lymphatic system, *Anatomical Record*, vol. 5, no. 8, 1911, p. 403, fig. 1, and p. 406.



Fig. 16 Successive drawings of the same growing lymphatic sprout in the dorsal fin expansion of the tail of a *hyla pickeringii* larva, to show the movements of the nuclear areas. Each of the numbers, 1 to 7, indicates the same nuclear area, which is dotted, in successive observations. In case of the division of a nucleus, the daughter areas are indicated as 1^a and 1^b . Enlarged 180 times.

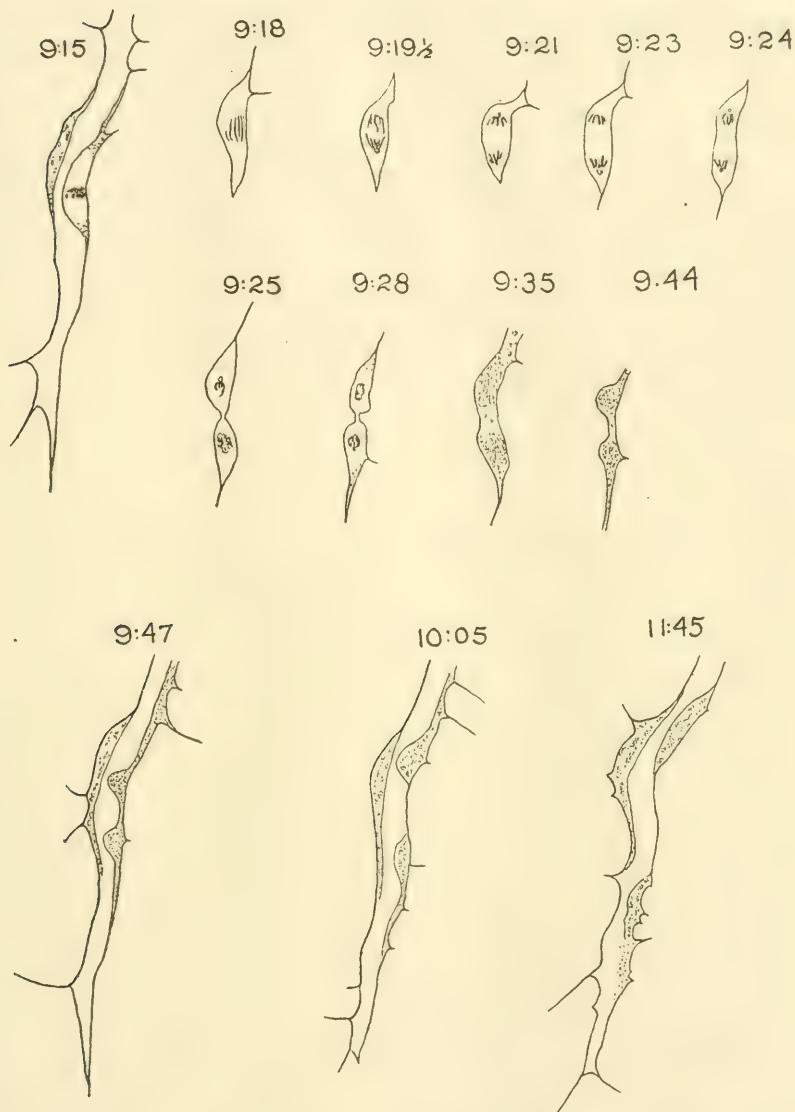


Fig. 17 Lymphatic sprout from the *hyla pickeringii* larva on which studies in figs. 1 to 14 were made, showing mitotic division of a nucleus. This division took place on May 5, while the larva was anesthetized with chloretone. The numbers show the time. From 9:18 to 9:44 inclusive, the dividing nuclear area alone is shown. Enlarged approximately 450 times.

they gradually resume the character of the non-dividing nuclear areas. During the division the wall of the lymphatic remains continuous.

Mitotic divisions have been observed in the main caudal lymph trunk as well as in the branches. There seems to be no definite proportion between the number of nuclear areas a branch may receive by in-wandering as compared with the increase by division. I have seen the first nuclear area in a sprout divide, one of the daughter nuclear areas remaining, and the other passing back to the main stem. On the other hand, I have seen as many as five nuclear areas wander into a branch.

Thus it is found to be possible to keep an accurate account of all the nuclear areas in a growing lymphatic sprout, from its very beginning. When this is done it is found that the sprout receives its nuclear areas in two ways—by the in-wandering along the wall of nuclear areas, which were present in the parent stem, and by mitotic division of those which have wandered in. Moreover all the nuclear areas which have been watched remain as nuclear areas of the lymphatic. I have not seen them form leucocytes, red blood cells, or mesenchyme cells. The sprout, then, receives its nuclear areas from the preexisting endothelium. That the protoplasm of the sprout is also derived from the preexisting endothelium was shown in an earlier paper.⁷

What, now, are the relations between the growing lymphatic and mesenchyme cells, which abound in the region which the sprout is invading? In all my studies it has been observed that the lymph sprout is so far from adding to itself these cells that it actually remains at as great a distance as possible from the main bodies and larger processes of the mesenchyme cells. At the tip of the sprout, long fine processes are sent out by the lymphatic in various directions. Occasionally such a thread pushes toward or even to the body of the mesenchyme cell. But in all instances, according to my observations, this process is eventually withdrawn and the path selected is, as said, midway between these cells (cf. the relations between lymph sprout and cell *J*, in figs.

⁷ E. R. Clark, 1. c. 1909.

4, 5, 6 and 7). It might be said that the mesenchyme cells keep the lymphatic at their finger tips, or that the lymphatic avoids the mesenchyme cells. I have seen a mesenchyme cell approach, in its wandering, a lymphatic lying across its course. Processes were sent out by the mesenchyme cell on both sides of the lymphatic so that the lymphatic lay within the *U*. In this case the processes of the mesenchyme cell remained away from the wall of the lymphatic, and the cell moved past on one side, gradually withdrawing the other arm of the *U*.

In summing up the results of these studies, there are certain facts on which I desire to lay especial emphasis. It will be recalled that the purpose of the investigation was to obtain a complete history of the growing lymphatic sprout and of the growing mesenchyme cell individually and side by side, in order to determine their relationship to one another—to find out whether the growth of the lymphatic is brought about by the addition of mesenchyme cells or of spaces lined by transformed mesenchyme cells, or whether the growth of the lymphatic is independent of the mesenchyme cells. The observations have furnished answers to these questions which are perfectly clear. Each of the two tissues has a characteristic independent life. The mesenchyme cell wanders and increases by mitotic division. It maintains throughout its identity as a mesenchyme cell, and is not transformed into lymphatic endothelium. The lymphatic grows by the sending out of fine protoplasmic processes which become definite lumen-containing sprouts. Nuclear areas in the sprout are provided by the in-wandering of nuclear areas from the main stem, and by mitotic division. New lymphatic protoplasm and nuclei, therefore, are formed by the extension of preexisting lymphatic endothelium. In its peripheral growth the lymphatic endothelium is not formed by the transformation of mesenchyme cells or blood vessels, nor does it give rise to mesenchyme cells or blood vessels, it is a specific independent tissue. Throughout its growth, the endothelial wall of the lymphatic capillary is closed, there are no open communications between the lumen of the lymphatic capillary and mesenchyme spaces.

THE ATTACHMENT OF MUSCLES TO THE EXOSKELETON IN THE CRAYFISH, AND THE STRUCTURE OF THE CRAYFISH EPIDERM

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FIVE FIGURES

According to Leydig, Janet, Hecht and Holmgren the striated muscles of Arthropods may be inserted without an intervening tendon. In such cases the muscle fibrils are continuous with the fibrous spongy network of the epithelial cells (Leydig), or the muscle is attached directly to the chitinous exoskeleton, the hypodermal epithelium being absent at the point of attachment (Janet, Hecht, Holmgren). The usual mode of attachment, however, is by means of an intervening tendon composed of straight fibrils whose length is equal to the thickness of the hypodermal layer within which they are located. The tendon fibrils are so numerous that little remains of the original hypodermal cells but a more or less distorted nucleus surrounded by a very small quantity of cytoplasm. Without ontogenetic studies it is, of course, almost impossible to determine whether these fibrils are within the hypodermal cells or between them. The former view is the one accepted by most authors, but Frenzel, Nicholas, Ide, Pantel and Hecht claim that the tendon fibrils are between the hypodermal cells. This is undoubtedly true in such cases as Holmgren describes for some of the Diptera, where the tendon is divided into several fine bundles of fibrils which pass between or spin around large epithelial cells having definite cell boundaries. However, this condition must be very exceptional, as most authors describe the hypodermis in the region of muscle insertion as being

composed largely of straight, stiff, coarse fibers which are so numerous that little remains of the original hypodermal cell except the nucleus, and cell limits are entirely obliterated.

A basement membrane or connective tissue 'Grenzlamella' between the hypodermal cells and the muscle fibrils, at the point of muscle insertion, is described by Claus, McMurrich, van Rees, Schneider, Holmgren and many other authors. Lécaillon, Snethlage and Riley also describe a basement membrane for the hypodermal cells, but in the region of muscle attachment it bends around and becomes continuous with the sarcolemma, and therefore it does not extend between the epithelium and the muscles. Claus states that it is a cuticular formation in *Branchipus* and *Artemia*, and that it may become chitinous. In some regions it is absent. In the Decapods, however, it is a true connective tissue 'Grenzlamella.' McMurrich and Holmgren also claim that it is merely a cuticular membrane formed by the epithelium, the former describing thickenings of the membrane (in terrestrial Isopods) at the cell boundaries from which supporting fibers originate and pass up into the cells. van Rees published figures which show that in *Musca vomitoria* the basement membrane is formed from long processes of the hypodermal cells which surround the muscle insertion. According to Emmel the first basement membrane in the regenerating claw of the lobster is a homogeneous cuticular formation of the ectodermal cells. A nucleated membrane is formed later, the origin of which Emmel was unable to determine. The intermediate membrane is of connective tissue origin according to Schneider, and Claus states that it is a connective tissue formation in the Decapods.

Claus found the basement membrane to be absent from some regions of *Branchipus* and *Artemia*, and Henneguy never saw it in the Insecta and claims that it is not present in other Arthropods, but that the Z lines of the muscle fibers give the appearance of a basement membrane. There is no basement or intermediate membrane in the Millipedes according to Duboseq. Snethlage finds no intermediate membrane at the point of insertion, but pigment granules and anastomosing muscle fibrils give the appearance of a basement membrane.

The muscles are attached to the epithelial cells and not directly to the tendon fibers according to List, Viallanes, Vitzou and Weisman. Stamm declares that the muscles are inserted to the basement membrane and that there are no fibrils passing through the membrane. Bertkau (in spiders) finds muscle fibers to be continuous with the tendon fibers, but he can always see a distinct dividing line between the epithelium and the muscle.

Leydig, Duboseq and Snethlage declare that the sarcoplasm is continuous with the protoplasm of the ectodermal epithelial cells, but this is denied by Holmgren, excepting for the vagina of *Sarcophaga*, where he found direct continuity between chitin matrix cells and the sarcoglia of muscles. This is explained as being the persisting embryonic connection between ectoderm and mesoderm which was described by Heatecote.

Tendon fibers and muscle fibers are directly continuous with one another according to Claus, Duboseq, Leydig, Lécaillon and Henneguy, but Riley declares that there is a splicing or fusion of the muscle fibers into the tendon fibers and no direct continuity between the two.

Many authors believe that the tendon fibrils are merely prolongations of the muscle cells. Frenzel, Nicolas, Ide, Pantel, Hecht and Holmgren locate the tendon fibers between the epithelial cells instead of within them. As the muscles approach the epithelium they are resolved into fine bundles of fibrils which pass between the epithelial cells to be inserted directly into the chitin. According to another scheme of Holmgren's the muscle fibrils penetrate the epithelial cells and are then inserted to the chitin. Nowikoff and Snethlage believe that both tendon and muscle are developed from ectoderm, so the continuity of the two types of fibrils is easily explained. Emmel found that in the regenerating lobster claw the new tendon fibrils are merely the non-striated ends of the muscle fibrils which are developed in an ectodermal syncytium. The striations may continue as far as the level of of the chitin-forming outer layer of this syncytium.

The tendon fibers are described by Claus as being very coarse, while Henneguy states that they are identical in appearance with fibrils in other parts of the epidermis where there are no

muscles. They correspond to M. Heidenhain's 'Tonofibrillen' which are formed in many epithelial cells, according to Maziarki and Labbé, and Janet observed that they may become chitinous. Lécaillon's observations show that a tendon may divide into two parts and that two tendons may fuse.

The tendon fibers are developed from the ectodermal epithelium according to Claus, Henneguy, Leydig, Duboseq, Maziarki, Labbé, Janet, Snethlage, Reichenbach, van Rees, Emmel, Riley and Bertkau, but Tullberg and Braun derive them from the connective tissue. Most of those authors who believe that the tendon fibers are prolongations of the muscle fibrils also believe that the muscles are products of the mesoderm, which compels the conclusion that the tendon fibers are also of mesodermal origin. This view, however, is not shared by Nowikoff and Snethlage, nor by Reed, Ost and Emmel. All of these authors derive the muscles and the epidermis from the ectoderm, the three last named basing their conclusions on a study of regenerating tissue.

A study of the development of the muscles has been made by Henneguy (insects), Reichenbach (crayfish) and vanRees (insects). The work of these authors shows the muscles to be of mesodermal origin, while the investigations of Snethlage (*Artemia salina*) furnish equally convincing proof for the ectodermal origin of the muscles in normal ontogeny. Reed, Ost and Emmel have shown that the regenerating muscles are of hypodermal origin. Henneguy studied insect embryos and found that the muscles were developed from mesodermal cells which were in contact with the hypodermis. Epithelial fibrils were differentiated at the same time that the muscle fibrils were formed, and the two became continuous. Snethlage's studies on developing *Artemia* muscles convinced him that they are formed from the ectoderm at the point of insertion. Development from the mesoderm would be impossible, because a mesoderm in the sense of a distinct germ layer does not exist in these forms. van Rees worked on the metamorphosis of *Musca vomitoria* and found that the basal portions of the hypodermal cells become spun out into fine processes which push the basal membrane away from the epithelial cells. These fine processes become the muscle tendons. Reichenbach con-

cluded from his studies on the development of the crayfish that the tendon fibrils are formed from the ectoderm, but that the muscles are developed from mesoderm. Emmel has shown that in the regenerating lobster claw the muscles and hypodermis are developed from an ectodermal syncytium. The tendon fibrils are the non-striated ends of the muscle fibrils. The basement membrane which is formed at first is also a product of the ectodermal syncytium.

The above gleanings from the literature show that the problem of muscle attachment in Arthropods can hardly be regarded as settled. We find a variety of opinions even on such a question as the exact mode of attachment of the muscle fibrils to the tendon fibrils, the solution of which depends chiefly on good technique and careful observation of sections of adult tissue. It seems, therefore, that any new observations in this field may be of value.

The following account of muscle attachment in the crayfish is based on a study of material from the histological collection of the department of Animal Biology of the University of Minnesota. The sections were made through the region of muscle attachment in the claw of a crayfish after the chitin had been stripped off. The fixation of the material is good. The sections are stained in a haematoxylin combination which gives a splendid differentiation of the finer cell structures.

The epiderm or hypodermis, as it is usually termed by entomologists, of the large claw of the crayfish may consist of one (figs. 4 and 5) or several (fig. 1) layers of extremely irregular cells which are so closely associated with one another that it is usually quite impossible to distinguish cell boundaries. A study of sections through regions located between the points of muscle attachment (figs. 1, 2, 4) shows very clearly that there are no cell boundaries, and that the hypodermal layer is composed of a protoplasmic syncytium in which the nuclei have a very irregular distribution, a condition which was described by Henneguy for various Arthropods and by Emmel in the regenerating claw of lobster. In some regions (fig. 1) the presence of small and large irregular spaces gives the syncytium the appearance of an extremely irregular protoplasmic network the strands of which are extremely

variable in size and shape. Vacuoles and small spaces are nearly always present, even in the thinner portions of the hypodermis (figs. 1 and 4). The nuclei all have about the same relative amount of chromatin, but their size and shape is subject to great variations. They may be round, irregular or oval, and their long axis may be in any plane. Very large nuclei are shown in figure 3 behind the tendon fibrils and at the right of figure 4, and figures 1 and 2 show the irregularities in the shape of the different nuclei.

Everywhere the hypodermis contains many sharply defined; homogeneous fibrils which take a wavy course through the syncytium, usually extending through several cell-territories. Sometimes they unite into bundles (fig. 1), but usually each fibril is independent. They show a general tendency towards an oblique course through the syncytium from its outer to its inner surface, or they may converge on the muscles, as is seen in figure 1, where the fibrils of the syncytium are all directed towards a small area on the bundles of striated muscle fibers shown in the lower right-hand portion of the figure. Occasionally, the fibers will run for some distance in the horizontal direction parallel to the outer surface, before they take the oblique course downwards to join the fiber bundles in the basal region of the syncytium (fig. 4). Figure 4 also shows considerable variation in the size and direction of the fibrils. In the upper right hand portion of the figure one sees many exceedingly fine fibrils whose course is more vertical than that of the other fibers. Some of the thicker fibers permit a considerable change of focus before they pass out of view, which indicates that some of them are bands or membranes and not fibers. Figure 2 shows a very interesting arrangement of the hypodermal fibers. If we examine the bundle of fibers in the basal portion of the hypodermis we notice that one fiber leaves this bundle, and if we follow it to the left we see that it gradually gets further away from the basal bundle until it very suddenly changes its direction. It passes upwards, bends around a hypodermal nucleus and can then be followed back to the right in the horizontal plane, where it is joined by several other fibers running in the same direction, some of which again pass downwards to join the basal bundle. That portion of the hypodermis which is some distance

away from muscle attachments may contain a great many horizontal fibers, some of which pass downwards to join the basal bundle of fibers (fig. 4). Others unite with vertical and oblique fibers which results in the formation of a network within the syncytium.

Supporting fibrils in the hypodermis are mentioned by Henne-guy, Maziarki, Labbé and Emmel, Maziarki and Labbé believing that they correspond to M. Heidenhain's 'Tonofibrillen' which are formed in many epithelial cells. In insects Lécaillon could see no other fibers in the epidermal epithelium but the tendon fibers which are continuous with the muscle fibrils. McMurrich found supporting fibrils passing up into the epithelial cells of the mid-gut of terrestrial Isopods from thickenings of the basement membrane.

In the crayfish the supporting fibrils of the hypodermis show a tendency to collect in the basal portion of the syncytium to form a horizontal layer of fibers which, when composed of many fibers not clearly differentiated by good technique, may have the appearance of a basement membrane. However, a good haematoxylin stain will show exactly what this 'basement membrane' is. Figures 1, 2 and 4 show clearly that most of the supporting fibrils of the hypodermis eventually reach the basal portion of the syncytium where they take a horizontal course. In some regions (fig. 1) the horizontal fibers form a dense bundle at the inner margin of the syncytium and thereby produce a definite limiting structure between the hypodermis and the underlying tissues, but in other cases (fig. 2) the basal fibers are not so closely associated with one another as to form a fibrous membrane. Figure 4 shows that the basal fibers may be widely separated from one another and that they may occupy the entire cell-territory between the lower level of the nucleus and inner margin of the syncytium. The same section (left-hand portion of figure 4) shows also that there may be about as many horizontal fibers above the nuclei as below them. In still other regions of the hypodermis horizontal fibers are entirely absent and there is absolutely no limiting structure of any kind between the hypodermal syncytium and the underlying connective tissue. In such places it is impossible to

determine the boundary between the hypodermis and the connective tissue, and the supporting fibrils are continuous from one tissue to the other. In the deeper portions of the connective tissue syncytium the fibrils pass to the margins of the syncytial strands, thus entering into the structure of true Leydig's cells of the first order. The fibers or bands are continuous from one Leydig cell to another but are always located at the margin of the cells. Some of the fibers join the outer adventitial membrane of the blood vessels the wall of which is composed of Leydig's cells which are so arranged that they form simple epithelial tubes which have a very definite inner and outer membrane. The inner membrane appears homogeneous, but it has the same structure and staining reactions as the fibers of the hypodermis and supporting tissue. The outer membrane is frequently fibrous, its fibers being continuous with those of the surrounding Leydig's cells.

That the hypodermis is continuous with the underlying supporting tissue is not a new observation. Leydig, in 1851, called attention to the similarity between Arthropod integument and connective substance and M. Braun, in 1875, states that it is impossible to separate the products of the ectoderm from those of the mesoderm in the crayfish, and many other authors have made the same observation. In view of these facts it is not surprising to find some of the fibrils from the supporting tissue joining the horizontal layer of hypodermal fibers (figures 1 and 2, of which *S. T.* is supporting tissue). We must admit, therefore, that it is quite possible that connective fibrils from the supporting tissue take part in the formation of the fibrous limiting layer where such a structure is formed but, on the other hand, it is also possible that the fibers which we see running down into the supporting substance are derived from the hypodermis. The presence of occasional flattened oval nuclei among the horizontal fibers has been interpreted by Schneider and others as proof for the existence of connective tissue in this region. Further speculation on this point is useless, especially so in view of the fact that we are by no means certain of the mesodermal origin of the supporting tissue or of the muscles in Arthropods. The fact remains, that in the crayfish, the layer of horizontal fibers is composed largely of hypo-

dermal fibers. There is no cuticular basement membrane, such as is described by McMurich, Holmgren and Emmel, and no connective tissue 'Grenzlamella' as stated by Claus and Schneider, although fibers from the supporting tissue may enter into the fibrous layer in the basal portion of the hypodermis.

Where the layer of basal fibers is well developed it may pass between the muscle and tendon fibrils in the region of muscle attachment (figs. 1 and 2), or it may bend downwards and run along the surface of the muscle or continue for some distance between the muscles (fig. 3). A horizontal layer of fibers between the muscles and their tendon fibers may also be produced by continuous branches of the tendon fibers themselves (fig. 5). This is probably what Snethlage saw when he stated that between the muscles and their tendon fibrils an apparent basement membrane is produced by anastomosing muscle fibrils and pigment granules. Small, highly refractive pigment granules are also found in the crayfish in this region, but they are seen only when the light is cut down. For the sake of clearness they have been omitted from the drawings.

Figures 1 and 4 show that bundles of fibers are not limited to the basal layer of horizontal fibers. At the outer border of the hypodermis these bundles are resolved into their individual fibers which spread out fan-shaped and probably penetrate the chitin. In the present investigation no attempt was made to determine the relation of the fibrils to the chitin, but many authors state that they are continued into the chitin.

Close study of the tendon fibers (*T. F.*, figs. 1, 2, 3, 5) shows that they are coarse, straight bundles of fibrils which seem to be identical with those which are found in other regions of the hypodermis. This is in accord with the findings of Henne-guy, but is contrary to Emmel. The outer ends of the tendon fibers, where they are attached to the chitin, are frequently spread out in such a way that they form a fan-shaped figure in sections. This is seen especially well in the isolated smaller bundles of fibers such as are shown in figure 1, *T. F.*' and on the left of figure 2. Here one sees that the fibrils correspond in size, structure and staining reactions to the supporting fibrils in other regions of the hypoder-

mis. The tendon fibers stain darker than the smallest fibers found in other parts of the epiderm, but not any darker than thick bundles of fibers (fig. 1). Where the tendon fibers are frayed out at the ends the staining reactions of their fine constituent fibrils are identical with those of fibrils in other parts of the hypoderm. It is probable, therefore, that the dark staining of the tendon fibers is due to their density, rather than to changes in their chemical nature.

A condition similar to that described above is seen also at the inner ends of the tendon fibers, where they are attached to the muscles (figs. 1, 3, 5). The fine inner branches resulting from the division of the coarser fibers into their constituent fibrils may follow a comparatively straight course, as in figure 3, or they may anastomose freely with neighboring fibrils at the lower border of the tendon, as in figure 5. The latter condition may produce a more or less horizontal layer or network of fibers composed of anastomosing branches of the tendon fibers (fig. 5). Such a structure might easily be mistaken for a basement membrane. Fibrils from the surrounding epidermal regions, and probably also from the supporting tissue, usually join with the branches of the tendon fibers in the formation of this layer (fig. 1). However, this is probably not the case in the region shown in figure 5, and certainly not in the parts shown in figure 3. The latter figure shows that a horizontal layer of fibers may be absent at the point of attachment of the muscle to its tendon, but even in this case there is more or less branching of the finer fibrils at their inner ends.

The exact mode of attachment of the muscles to their tendons is shown in figures 1, 3, and 5. In figures 1 and 3 we see that the fine branches of the tendon fibers are continued down into the muscle for a variable distance, and that the muscle fibrils run up between these processes. Figure 1 also shows that fibers from the horizontal layer which are derived from other regions of the hypodermis may penetrate the muscle for some distance. The long fiber on the right of the figure is probably derived from this source. There are no horizontal fibers in the greater part of figure 3, and therefore the muscle attachment here is entirely by means of fine branches of the tendon fibers. In figure 5 most of the fibrils

which project down into the muscle are derived from the network which is produced by anastomosis of tendon fibrils at the base of the tendon. Some of the fibrils pass down into the muscle for a considerable distance. The 'splicing' or 'dove-tailing' of the muscle fibrils and tendon fibrils is seen very clearly in this figure and in figure 1.

The findings of previous workers in regard to the union of tendon and muscle in Arthropods have been presented in the first part of this paper and it is unnecessary to restate them here. Riley seems to be the only author who has observed anything like the process described here. According to Riley, ". . . there occurs a slicing or fusion of the two types of fibrils, the basement membrane being lacking at the point of contact." If the network or layer of fibers at the base of the tendon and in the lower portion of the hypodermis is to be interpreted as a basement membrane, then Riley's statement in regard to this structure is not correct for the crayfish, as is seen by an examination of figures 1 and 5. However, the writer can see no reason for calling this structure a basement membrane. The variations to which it is subject are shown in the five figures presented here, and it hardly seems reasonable to call such an indefinite structure a basement membrane.

For the crayfish it seems almost certain that the tendon fibers are within the hypodermal cells and not between them, as is claimed by Frenzel, Nicolas, Ide, Pantel and Hecht. The fact that the tendon fibers seem to be bundles of ordinary supporting fibrils is in favor of the former view, and figures 1, 3 and 5 can be interpreted in no other light. The fibers occupy the greater part of the cell, but a small quantity of cytoplasm can always be seen between them, and the original hypodermal nuclei are still to be seen. The final solution of this question will depend on ontogenetic studies, but for the present the view presented here seems to be the most reasonable one.

The origin of the muscles in the Arthropoda is still being debated, some authors claiming that they are of mesodermal origin, others that they are derived from ectoderm. It is very likely that they may be derived from both sources, which is probably also true

of some vertebrates, Goronowitsch having shown that in birds the musculature of the visceral arches is derived from cells of the neural crest and from the outer ectoderm. Julia Platt does not admit the development of muscles from wandering extoderm cells in *Necturus*, but she does claim that the branchial cartilages and the anterior portions of the trabecular bars are developed from ectoderm, while Goronowitsch claims that in birds a portion of the mesenchyme, cutis and skull in the region of the midbrain are formed from ectoderm. According to Kastschenko the mesenchyme is derived from all three germ layers, from cells which are not used up in the formation of epithelial structures, and v. Kupffer derives the branchial cartilages of *Petromyzon* from the deeper layers of the ectoderm.

Facts like these show that we must be careful in our statements regarding the origin of various structures from certain germ layers until we have exact ontogenetic studies of those structures, and this is especially true of the invertebrates.

The peculiar arrangement which provides for union between muscle and tendon fibrils in the crayfish suggests independent origin and secondary fusion of those structures, but the ontogenetic proofs for this are lacking. In any case, the method of union between the two types of fibrils remains quite different from what it is in vertebrates. In the latter there is direct continuity between muscle and tendon fibrils, as has been recently proven by Oskar Schultze¹ for man and several groups of vertebrates. His results have been confirmed by other investigators (see Schultze's Leipzig paper for further literature on the subject), and the writer can testify that his preparations show all that is claimed for them. That many authors believe that the same conditions obtain in Arthropods has already been pointed out. However, the present investigation does not warrant this conclusion for the crayfish. Here the ends of the muscle fibrils are surrounded by fine branches of the tendon fibrils, some of which may pass in between the muscle fibrils for a considerable distance.

¹ Verhdl. der Phys.-Med. Gesellsch. zu Würzburg N. F. Bd. 41 and Verhdl. der Anat. Gesellsch, 25. Vers., Leipzig, 1911.

Coagulated blood plasma is found in some of the spaces of the hypodermal syncytium and in occasional spaces which occur in the layer of horizontal fibers at the base of the epiderm. This indicates that the vascular circulation is in very close relation with the hypodermis.

SUMMARY

The hypodermis of the crayfish consists of a protoplasmic syncytium containing one or more layers of nuclei which have a very irregular distribution within the syncytium.

Supporting fibers and fibrils are found everywhere within the syncytium. In some regions they all take about the same course and eventually reach the basal portion of the syncytium where they run in a horizontal direction.

The basal layer of fibers shows great variation in the number of fibers entering into its composition and in their proximity to each other. In some cases the basal fibers occupy the entire territory between the nuclei and the lower border of the syncytium, and in others this layer is absent altogether. On account of these variations this layer of fibers can not be classified as a true basement or intermediate membrane.

Fibers from the supporting tissue are continuous with those of the hypoderm and some of them join the basal layer of hypodermal fibers which, in the regions of muscle attachment, usually pass between the ends of the muscle fibrils and the tendon fibers by means of which the muscles are attached to the chitinous exoskeleton.

The tendon fibers are bundles of fine fibrils, similar to those found in other regions of the epiderm. At their inner ends the tendon fibers are resolved into their constituent fibrils which usually anastomose with neighboring fibrils in such a way as to produce a network at the base of the tendon. Fibers from other regions of the hypoderm and from the supporting tissue frequently join this network (fig. 1).

Tendon fibrils, and fibrils from the intermediate network or basal layer of hypodermal and supporting tissue fibrils penetrate

the muscle for variable distances in such a way that the outer ends of the muscle fibrils are surrounded by them.

Muscle fibrils and tendon fibrils are not directly continuous with one another. The muscle is 'dove-tailed' or 'spliced' into its tendon.

The evidence obtained from a study of the adult structures is in favor of the view that the tendon fibers are located within the hypodermal cells and not between them.

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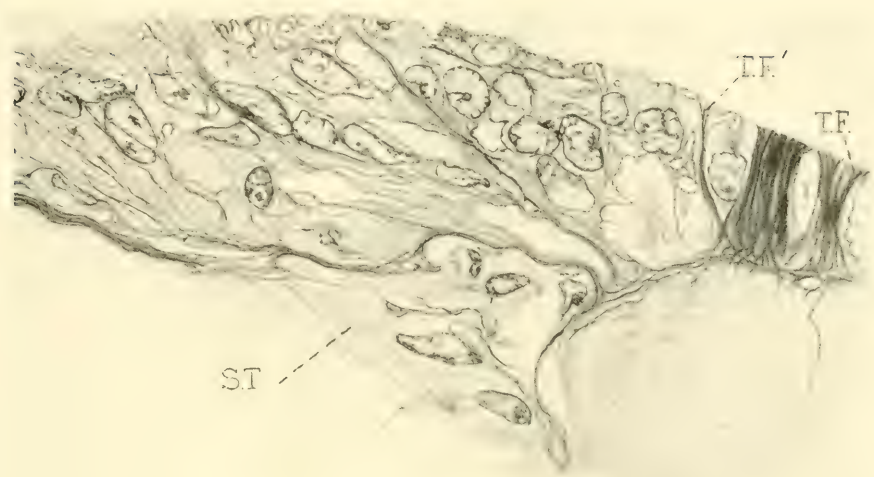
All drawings were made with the camera lucida under Zeiss apochrom. obj. 2 mm. and compens. ocular 6, drawing board at the height of the stand.

PLATE 1

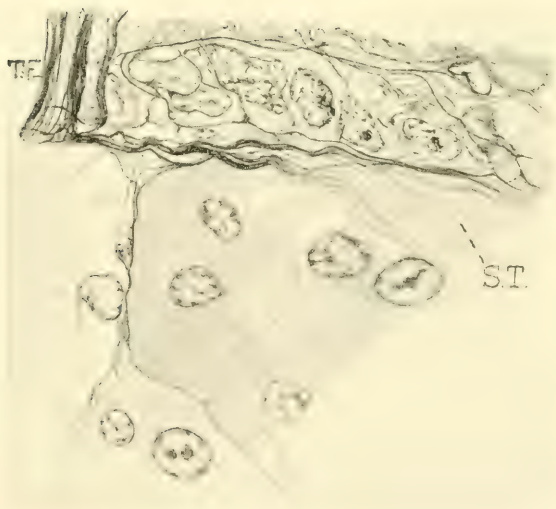
EXPLANATION OF FIGURES

1 Striated muscle and its tendon on the right, thick layer of hypodermal syncytium on the left. Notice the numerous supporting fibrils within the syncytium, the irregular distribution of its nuclei and the irregular spaces. A horizontal layer of fibrils in the basal portion of the syncytium passes between the muscle and its tendon fibers. The figure shows that the fibrils of this layer are derived from the hypodermis and also from the supporting tissue, *S. T.* Tendon fibrils and fibers from the horizontal layer penetrate the muscle for varying distances.

2 Shows an arrangement of supporting fibers in the hypodermal syncytium which is quite different from that shown in figure 1. Horizontal fibers are found both above and below the nuclei. The inner layer of horizontal fibers occupies almost the entire cell-territory between the nuclei and the inner border of the syncytium. This arrangement of the fibres shows that the basal layer of horizontal fibers does not form a true basement or intermediate membrane.



1



2

Helen A. Sanborn, del.

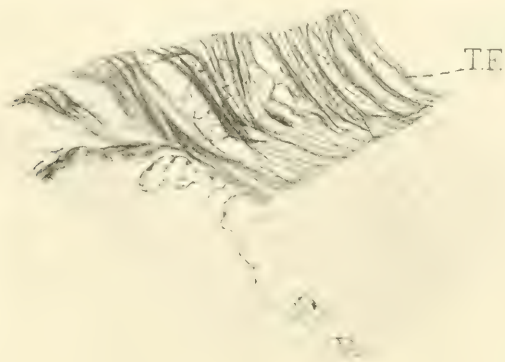
PLATE 2

EXPLANATION OF FIGURES

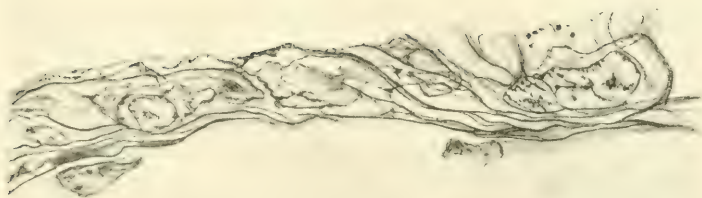
3 Very long tendon fibers which show a rather loose arrangement. The tendon fibers branch at their inner ends, the fine branches surrounding the ends of the muscle fibrils. Large nucleus of the hypodermal syncytium back of the tendon fibers. This figure also shows that the horizontal layer of fibers may be absent at the point of attachment of the muscle to its tendon.

4 A clear demonstration of the fact that the basal layer of horizontal fibers is composed largely of fibers derived from the hypodermal syncytium. In this case the basal layer can hardly be interpreted as a true basement membrane or as a connective tissue intermediate membrane. Horizontal fibers are seen both above and below the nuclei. Those above eventually join the lower layer which occupies most of the cell-territory between the nuclei and the inner border of the epiderm.

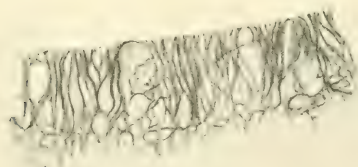
5 Striated muscle and its tendon. This section is especially favorable because the tendon fibers show a very loose arrangement which provides for a clear demonstration of their relation to the muscle fibrils. The network of fibers at the base of the tendon is formed by anastomosing branches of the tendon fibers. Tendon fibrils and fibrils derived from the network penetrate the muscle between the ends of the muscle fibrils. This arrangement shows that the muscles are 'spliced' or 'dove-tailed' into their tendons, and that there is no direct continuity between the tendon and muscle fibrils.



3



4



5

Helen A. Sanborn, del.

THE DEVELOPMENT OF THE THORACIC DUCT IN THE PIG

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THIRTY-FIVE FIGURES (FIVE COLORED PLATES)¹

I. INTRODUCTION

At the present day the question of the origin of lymphatics has become one of the most interesting problems and is holding the attention of perhaps as great a number of investigators as any other problem in the field of embryology and anatomy. This is partly due to the fact that the lymphatic system as a whole is the last of the organ-systems to be taken up for more thorough investigation, and partly it is the result of the impetus given by modern physiology which has emphasized the question of the relationship between lymphatics and blood vessels and their functional significance in the economy of the organism. Although this problem has been attacked at various times in the history of anatomy, relatively few important advances have been made, and it is only recently during the last decade that it has been attacked with renewed vigor and has been pushed to the critical point of its solution. The question of the individuality of a lymphatic, especially, has developed a most animated controversy between two schools diametrically opposed in their contentions. One of these maintains that all lymphatic channels are a direct product of the venous system, and the other that they arise independently of it, and that if they do enter into certain structural venous relations during their genesis such relations are purely of a secondary character.

¹ Expense of illustrations partly borne by author.

In 1909 Sabin² published a paper on the origin of the lymphatic system in the pig which reinforced and extended the theory of Langer³ and Ranvier,⁴ that lymphatic vessels arise from the veins by a process of sprouting and centrifugal growth. Because her results and conclusions did not present fully all of the evidence or agree with the results of later investigations, Professor McClure suggested to the writer the expediency of repeating her work on the same kind of material, namely, pig embryos, but restricting this research to one lymphatic channel and concentrating upon it alone all attention. The thoracic duct was selected on account of its size, importance, and definite position in the body, thus serving better as a test or control than perhaps any other lymph channel.

For the completion of this work, carried on during the last two years, I am indebted to Professor McClure for his advice and valuable criticisms, and to Dr. A. G. Brown of Columbia University and to Mr. Charles F. Silvester for their hints in the preparation of the microphotographs. I acknowledge my gratitude to Professor Houser for permitting me to use the laboratory at the Iowa State University during the summer of 1910. My thanks are also due to Professor Sabin for the use of series 23a of the Johns Hopkins University Embryological Collection, and for the privilege of publishing any information or evidence which might be derived from this series.

II. MATERIAL AND TECHNIC

Most of the embryos used in this work were fixed in Zenker's fluid and the sections stained on the slide with Delafield's haematoxylin and orange-G. Sabin's series 23a was preserved in the same fixative but stained with Congo red. A few embryos were

² Florence R. Sabin: On the origin of the lymphatic system from the veins, and the development of the lymph hearts and thoracic duct in the pig. *Am. Jour. Anat.*, vol. 1, 1902, pp. 367-389.

³ C. Langer: Ueber das Lymphgefäß-systems des Frosches. *Sitzb. d. Akad. d. Wissensch.*, Bd., 57, 1, Abth., 1868.

⁴ L. Ranvier: Morphologie et développement des vaisseaux lymphatiques chez les mammifères. *Comptes Rendus*, 1895, 1896. *Archives d'Anatomie Microscopique*, tome 1, 1897.

fixed in picro-sublimate and chrom-aceto-formaldehyde, and several others were stained with borax carmine in toto and counterstained with picric acid or blue de Lyon. Picro-sublimate is very unsatisfactory in its preservation of tissue and should not be used. For excellency of fixation and differentiation of vascular structures the first method mentioned proved by far the best. Not only did the sections show a beautiful transparency of color and a strong contrast when this method was carefully followed in detail, but they also produced the most favorable microphotographs.

A few embryos were injected with India ink by the writer. Series 23a was prepared and injected by Professor Sabin and serves as an excellent critical stage in the development of the thoracic duct.

Table 1 which represents only a partial catalogue of the Princeton Collection of pig embryos enumerates the series studied and reconstructed. They are arranged and grouped according to the developmental status of the thoracic duct region in each, and therefore certain embryos precede others of slightly lesser length. On the whole, however, this system of gradation corresponds very closely to gradations by length, the little inconsistencies here and there being of trivial account when we consider that measurement can be at most of only approximate accuracy, and that fluctuations in growth are not at all infrequent.

The length of each embryo, excepting Sabin's series 23a, was obtained after fixation, when the danger of possible mutilation to the embryo is least, and represents the crown-rump measurement, viz., the distance between the crown of the head and the base of the tail. Sabin's series being measured before fixation is consequently longer; but when it is considered that the processes of fixation, hardening and dehydration reduce the absolute length of such an embryo by 1 or 2 mm., this discrepancy in size disappears and it is seen to fit in smoothly with related series.

All of the embryos were sectioned transversely to 15 and 20 micra in thickness. The significant sections of the series selected for reconstruction were carefully drawn with the aid of the Edinger drawing apparatus, the details then confirmed by the high power

of the microscope, and the wax models made after a slight modification of Born's method. In this way the regions of immediate interest in nine series were reproduced at a magnification of 100 diameters, excepting series 69 which was magnified to 50 diameters. Besides these wax-models, a number of graphic reconstructions

TABLE 1
List of material examined

SERIES NO.	LENGTH OF EMBRYOS	REMARKS
	<i>mm.</i>	
214	14	
215	14	
211	15	
210	15	
212	16	
151	16	
216	16	
217	16	
Unnumbered	16	Injected
150	18	Reconstructed in wax, $\times 100$
167	17	Reconstructed graphically, $\times 100$
141	17	
168	19	Reconstructed in wax, $\times 100$
101	19	
104	18	
194	20	Reconstructed in wax, $\times 100$
106	20	
102	20	
193	20	Reconstructed graphically, $\times 100$
186	21	
227	20	
Unnumbered	21	
23a	23	From the Johns Hopkins U. Emb. Coll.; injected; reconst. in wax, $\times 100$
103	21	
191	21	Reconstructed in wax, $\times 100$
105	22	Posterior region reconstructed in wax, $\times 100$
192	21.5	Reconstructed in wax, $\times 100$
Unnumbered	23	Injected
67	23	Posterior region reconstructed in wax, $\times 100$
Unnumbered	24	Injected
69	26	Anterior region reconstructed in wax, $\times 100$
Unnumbered	28	

were carefully made, but on account of the more restricted accuracy of this method and the probable errors in the expression of relations and proportions none were used as plate figures.

The series labelled 'unnumbered' represent incomplete series, embryos of which only the anterior two-thirds or middle regions were sectioned.

III: REVIEW OF LITERATURE

It is needless to survey all of the former investigations which have been concerned with the genesis of the lymphatic system; suffice it to say that the fundamental question in all of them has been: Do the lymphatics arise from the veins, or are they a direct product of the mesenchyme? Only the more recent literature bearing on this query and intimately related to the writer's own observations will be cited and discussed here.⁵

In 1902 Sabin published her first work on the development of the lymphatic system.⁶ By the method of injection she found that all lymph vessels, both peripheral and systemic, arise at four centers, two anterior and two posterior, and that they invade the skin as well as the deeper-lying regions of the embryo by a process of centrifugal growth. In other words, consecutive injected stages will show the channels springing apparently as a few simple sprouts from these points of radiation and then gradually growing longer and branching in an intricate manner until they have spread throughout the whole body. The two anterior centers, situated one on each side of the neck in the fork of the jugulars, and the posterior ones, inguinal in position, represent the locations of the anterior and posterior lymph hearts, respectively. With the aid of a series of clear diagrams she has mapped out the general course of lymphatic development. Unfortunately, how-

⁵ For a comprehensive list of the literature bearing on the development of the lymphatic system, the reader is referred to the following two papers: George S. Huntington: *Die Entwicklung des lymphatischen Systems der Vertebraten vom Standpunkte der Phylogenese des Gefäss-systems*. *Anat. Anz.*, Bd. 39, 1911. Florence R. Sabin: A critical study of the evidence presented in several recent articles on the development of the lymphatic system. *Anat. Rec.*, vol. 5, no. 9, 1911.

⁶ Florence R. Sabin: 1902; loc cit.

ever, she did not describe or figure the details or factors of this growth, except to say that the lymph vessels at first bud from the veins and thereby derive their endothelium from them and then continue to grow distally apparently by the proliferation of their cells. The sprouting and elongation of the thoracic duct is indicated in three diagrams constructed from embryos of 20, 27, and 30 mm. In the first of these schemes the right lymphatic and thoracic ducts are seen as two short caudal extensions from the jugular lymph sacs. In the second, they have become longer and the left one or thoracic duct has divided into two branches, one of which passes to the right side of the aorta as the rudiment of the right thoracic duct, and the other remains on the left side as the left duct. In the diagram of the 30 mm. embryo these two branches have extended far back and have established continuity with the cisterna chyli and the posterior lymph hearts, thus completing the thoracic duct system.

A few years later, in 1906, F. T. Lewis made public a short account of the development of the lymphatic system in rabbit embryos.⁷ His results are of great interest because they represent a new conception of the genesis of the lymphatics, being neither identical with the centrifugal growth theory of Sabin nor with the theory of their direct mesenchymal origin, but in a sense standing between these two. Furthermore, he was the first investigator to determine the principle involved in the formation of the lymph sacs and to point out certain definite events preparatory to the completion of the thoracic duct in the mammalian embryo. In this studies on the transformation of the venous system in the posterior regions of rabbit embryos, he noticed that portions of the subcardinal veins became isolated and seemingly converted into lymphatic vessels. He found more and more of these so-called detached 'lymphatics,' and led by this suggestion, he took up a more systematic investigation of the pathways of the larger systemic lymphatics of the body. He made a number of serial graphic reconstructions and thereby brought to light some very interesting results. He observed that the jugular lymph

⁷ Frederic T. Lewis: The development of the lymphatic system in rabbits. *Am. Jour. Anat.*, vol. 5, 1905, pp. 95-111.

hearts arise by the coalescence along the internal jugular vein of several venous outgrowths which become detached to form a large isolated sac. A similar process was observed in the posterior part of the body in the region of the subcardinal and mesenteric veins. He also discovered a chain of discontinuous 'lymphatic spaces' or endothelial-lined anlagen, apparently detached venous outgrowths, situated along the azygos veins and in the path of the future thoracic duct. From their position and consecutive arrangement he concluded that they fuse with one another and the jugular and mesenteric sacs and thus produce the continuity of the duct. To quote his own words: "The study of the specimens seems to show that the lymphatics along the aorta (thoracic ducts) are derived in part from the azygos veins; below from the subcardinal; and above from the jugular sacs." It is also of importance to state here that the 'lymphatic spaces' which he described are 'scarcely distinguishable from blood vessels.'

In 1907, shortly after the work of Lewis, Huntington and McClure made a more detailed and extensive study of the development of the jugular lymph sacs and confirmed in the main his results.⁸ They had formerly believed that the sacs arise by the formation, enlargement and fusion of perivascular spaces,⁹ but now, from the data of a large number of beautiful and accurate wax reconstructions of cat embryos, they established the opinion that they come from the precardinal and in part from the postcardinal veins by the confluence of a series of outgrowths or derivatives which they called veno-lymphatics, as suggesting their venous origin and their subsequent transformation into a lymphatic structure. For a short time McClure also carried this conception to the developing thoracic duct,¹⁰ agreeing with Lewis in the formation of a chain of discontinuous anlagen along

⁸ George S. Huntington and Charles F. W. McClure: The anatomy and development of the jugular lymph sac in the domestic cat. *Anat. Rec.* vol. 2, 1908, pp. 1-18. *Am. Jour. Anat.*, vol. 10, no. 2, April, 1910, pp. 177-311.

⁹ George S. Huntington and Charles F. W. McClure: The development of the main lymph channels of the cat in their relation to the venous system. *Am. Jour. Anat.*, vol. 6, 1907. *Abstr. Anat. Rec.*, vol. 1, pp. 36-41.

¹⁰ Charles F. W. McClure: The development of the thoracic and right lymphatic ducts in the domestic cat. *Anat. Anz.*, Bd. 32, nos. 21 and 22, 1908, pp. 534.

the azygos veins and derived from them, but he later withdrew this view since it was based on the study of an insufficient number of critical stages.

Concerning all lymphatic vessels, not including the lymph hearts, Huntington¹¹ maintained the theory advanced jointly by McClure and himself in 1906, that lymphatics have their origin in the fusion of extra-intimal spaces which arise irregularly and disjointly along primitive temporary venous channels.¹² Thus he says:

The peripheral general lymphatic channels appear to be developed by confluence of spaces independent of the venous system, although closely associated with the same. The histological picture presented by them differs radically from that of the jugular veno-lymphatic derivatives. They begin as minute extravascular vacuoles closely applied to the surface of the veins which they accompany. They enlarge as the lumen of the veins diminishes. They become confluent with each other but they never from their first inception contain red blood cells, nor do they, as far as I have been able to ascertain in numerous carefully studied series of excellent preservation and fixation, communicate with the blood channels.

In 1908, Sabin published a short paper¹³ in which she reviews the several positions held relative to the genesis of lymphatic channels and attempts to turn the evidence in favor of the centrifugal growth theory. Concerning Lewis' multiple anlagen she says:

Since these spaces are lined with a definite endothelium, they form a much more serious obstacle to the theory of growth of the lymphatics from the endothelium of the veins than the more indefinite spaces to be found in earlier embryos, and I cannot but think that if these multiple endothelial-lined isolated spaces do exist along the veins in the later stages, they would form serious evidence against the theory of the origin of the lymphatics from the veins. Or at least if the lymphatics, in their growth, do pick up isolated endothelial-lined spaces, we shall again be left without a clue as to the origin of the lymphatic system.

¹¹ George S. Huntington: The genetic interpretation of the development of the mammalian lymphatic system. *Anat. Rec.*, vol. 2, 1908, pp. 19-45.

¹² This theory was presented by Huntington and McClure before the Association of American Anatomists in 1906 and published as a preliminary account in the *Anatomical Record* no. 3 and in the *American Journal of Anatomy*, vol. 6, 1907.

¹³ Florence R. Sabin: Further evidence on the origin of the lymphatic endothelium from the endothelium of the blood vascular system. *Anat. Rec.*, vol. 2, 1908, pp. 46-54.

However, she firmly believes that the 'lymphatic anlagen' of Lewis appear isolated only in the study of serial sections, and that their continuity can be demonstrated by the method of injection. In other words, "in complete injections there are no vessels which have not received the injecting mass," but "in partial injections and uninjected specimens there are endothelial-lined vessels" which appear to be broken up into segments, so that continuity "can be traced only with difficulty or not at all." In this same article she admits the presence of true mesenchymal spaces "which undoubtedly contain lymph," but tacitly assumes that they "are to be excluded from the lymphatic system morphologically." They are isolated and cannot be injected and they do not possess a clearly defined intima.

A year later, in 1909, Sabin published her observations on the development of the lymphatic system in human embryos.¹⁴ In this work she reaches and emphasizes essentially the same points as in her previous investigations. In the case of the thoracic duct, however, she hesitates to take a definite position. She believes that it originates as outgrowths of the jugular lymph sac and cisterna chyli, but she states in this connection that the "thoracic duct has proved to be the most difficult part of the lymphatic system to work out for this reason, we have not yet found a way to inject it in early stages and uninjected sections are not adequate." Further:

The question is, does the duct develop from multiple anlagen from the azygos veins for which there is no proof except that lymphatic vessels can be seen in sections adjacent to these veins, or does the duct grow from the two sacs, the cisterna chyli and the jugular one. For the second view the evidence is also weak, it consists in this, that other lymph ducts wherever we can study them grow from the sacs; and secondly in pig embryos and in human embryos one can trace a duct forward from the cisterna chyli and caudalward from the jugular sac, and in later stages these two ducts have joined. The weakness of this evidence lies in the fact that in earlier stages the picture is always liable to be confused by Lewis' multiple anlagen.

¹⁴ Florence R. Sabin: On the development of the lymphatic system in human embryos, with a consideration of the morphology of the system as a whole. *Am. Jour. Anat.*, vol. 9, 1909, pp. 43-90.

In 1910 Huntington¹⁵ and McClure¹⁶ read two papers at the International Congress of Anatomists at Brussels and, on the basis of a study of cat embryos, presented striking evidence for the theory, that the lymph ducts of the body are developed by the confluence of mesenchymal spaces which are largely extra-intimal, that is, formed around the lumen of an embryonic venous channel which subsequently disappears completely. Furthermore, they drew the distinction very clearly that the lymphatic system of mammals may be divided into two morphological components: the lymph ducts which arise as indicated, and the lymph hearts which form the connecting segment between the systemic lymphatics and the veins and are transformed from a venous plexus derived from the veins in their respective regions. Concerning the former, which at present are of the greatest interest, Huntington says:

Die Lymphgefäße des ganzen Körpers entstehen durch den Zusammenfluss einer grossen Anzahl von Hohlräumen, welche sich intercellulär in Mesoderm entwickeln, in sehr genauer Anpassung an die Wand der Embryonalen venösen Bahnen und in ganz derselben Weise wie die ersten Anlagen des Blutgefäss-systems, aber unabhängig vom demselben. Das Endothel, welches diese Hohlräume die ersten lymphatischen Anlagen auskleidet, ist von Anfang an unabhängig vom Endothel der Blutbahnen und entwickelt sich mit dem ersten Auftauchen der lymphatischen Hohlräume aus den indifferenten Mesodermalzellen, welche diese Hohlräume begrenzen. Mit anderen Worten, das lymphatische Endothel hat dieselbe genetische Herkunft wie das Endothel der Blutgefäße, nämlich es besteht aus modifizierten Mesodermalzellen, welche in die Wandung der intercellulären Hohlräume eintreten. Die erste Stufe des histogenetischen Verlaufes ist ganz die gleiche, ob nun das resultierende Hohlraumssystem in der Folge der lymphatischen oder der hämalen Abteilung des Gefäss-systems zugeteilt wird. Es gibt demnach zwei Generationen der embryonalen vaskulären Endothelzelle, eine lymphatische und eine hämale. Beide entstehen auf gleiche Weise und infolge gleicher genetischer Einflüsse aus indifferenten Mesodermalzellen. Beide sind vom Anfang des Vorganges an unabhängig voneinander.

¹⁵ George S. Huntington: Ueber die Histogenese des lymphatischen Systems beim Säuger-embryo. Verhandl. d. Anat. Gesellsch., Bd. 24, 1910.

¹⁶ Charles F. W. McClure: The extra-intimal theory and the development of the mesenteric lymphatics in the domestic cat. Verhandl. d. Anat. Gesellsch., Bd. 24, 1910.

In order to emphasize the principle involved in the extra-intimal development of the mammalian lymphatics, Huntington selected the thoracic duct as an example for the reason that the histogenetic processes which enter into its inception and completion are clearly expressed and easily followed, and also because the duct retains a more definite and constant position relative to surrounding structures than perhaps any other lymph channel. He observed that the continuity of the thoracic ducts is realized by the confluence of a large number of spaces which have sprung from the mesenchyme immediately in contact with decadent venous channels. Their first appearance is as numerous intercellular isolated fissures which then coalesce to form larger spaces, and these in turn become confluent to produce the continuous vessels. Lined with undifferentiated tissue cells at their beginning, they gradually assume the flattened and delicate endothelium of the adult lymphatic.

McClure has described the same process of development in the formation of the mesenteric lymphatics of the cat.¹⁷ He showed that at a certain period, a plexus of veins situated in the dorsal mesentery becomes detached from the postcava and soon after manifests signs of atrophy. With the aid of several clear microphotographs he further pointed out that the mesenteric lymphatics follow topographically, or better, appropriate these abandoned venous channels by a process of extra-intimal replacement. The haemal endothelium collapses and large mesenchymal spaces appear around it. In this connection he states:

These lymph spaces which lie external to the intima of the veins gradually encroach upon the territory formerly occupied by the veins and finally fill it completely; the result being that the original intima of the vein, no longer serving in the capacity of lining a functional venous channel, gradually degenerates and disappears. Traces of this intima can often be observed, however, in older embryos, clinging to the walls of the lymph channels within which a new lymphatic intima has been established.

That these pictures are real, and not artifacts induced by poor preservation of tissue, is conclusively shown by the fact that they

¹⁷ Charles F. W. McClure: 1910; loc. cit.

occur only at a definite period and place, and only in connection with those venous channels which have become detached from the main venous trunks and no longer serve in the economy of the blood vascular system.

Last year there appeared in monograph form the first two parts of Huntington's investigations on the anatomy and development of the systemic lymphatic vessels in the cat.¹⁸ Besides its great detail, the work is profusely illustrated with convincing microphotographs and reconstructions and is a very positive and elaborate confirmation of the theory of the direct mesenchymal origin of all lymph ducts. Once more he emphasizes sharply the analogy between the blood vascular and lymphatic systems in their earliest anlagen, both beginning their history in a similar manner and in the same soil. The first blood vessels arise in and amongst the strands and 'blood islands' of the mesoderm as intercellular clefts and fissures which enlarge, elongate and flow together to create a network of intercommunicating channels. Their boundaries at first are the unspecialized and cuboidal mesodermal cells among which they lie. The fluid which fills their cavities and which is perhaps secreted by these cells is evidently under a certain pressure and exerts its influence in the modification of the immediate or limiting walls into a vascular endothelium. The cells by a mechanical adaptation to this pressure lose their cuboidal form and become flattened and scale-like. Likewise the lymphatic anlagen begin as intercellular spaces and enlarge, elongate and coalesce into continuous vessels, and like the intima of the blood vascular anlagen their intima is a differentiation of the cells among which they are formed.

After such general considerations, Huntington enters into a very complete description of the development of the thoracic duct. Because a résumé of this history, as determined by him, has already been given in the review of an earlier paper, it need not be repeated here. Suffice it to say that nowhere has he found the

¹⁸ George S. Huntington: The anatomy and development of the systemic lymphatic vessels in the domestic cat. Part I. The development of the systemic lymphatics in their relation to the blood vascular system. Part II. The development of the pre-azygos and azygos segments of the thoracic duct. *Memoirs of the Wistar Institute of Anatomy and Biology*, May, 1911.

slightest evidence for 'centrifugal growth' as the fundamental principle in the genesis of the thoracic duct, nor for its origin from multiple venous anlagen.

In her most recent paper Sabin¹⁹ takes a position plainly at variance with her earlier view of the development of the thoracic duct, in that she restricts to a considerable degree the importance of centrifugal growth by budding as the active principle or factor in its formation. After briefly mentioning the conditions found by her in two 23 mm. embryos and one measuring 25 mm., she says:

It is not possible to set limits to the transformation of veins into lymphatics making the cisterna chyli and thoracic duct, for by comparing the two specimens measuring 23 mm. it can be seen that vessels which are clearly branches of the azygos veins in the one specimen do not seem to connect with the vein in the other. The thoracic duct develops in part as a down growth of the jugular sac and in part, especially its dilated portion or cisterna chyli, as a direct transformation of the branches of the azygos veins.

This quotation would seem to indicate that she now believes that the longer part of the thoracic duct is produced as a caudal extension of the jugular lymph sac alone, and not, as she formerly held, from two growing sprouts which subsequently meet, one of them derived from the jugular sac, and the other from the cisterna chyli as an extension cephalad. Her failure to mention either the absence or presence of this last or second sprout, which she claimed to have found in her earlier investigations on the origin of the thoracic duct in human and pig embryos, confirms strongly enough her change of view in this respect.

IV. OBSERVATIONS AND DISCUSSION

It is clear now, that there are three distinct views in the field concerning the development of lymphatic vessels:

1. They spring from the veins at four centers of radiation and by continuous elongation, centrifugal growth and branching invade practically the entire body.

¹⁹ Florence R. Sabin: A critical study of the evidence presented in several recent articles on the development of the lymphatic system. *Anat. Rec.*, vol. 5, no. 9, 1911.

2. They are derived from the embryonic venous system either by a direct transformation of certain of its channels or by the fusion of multiple derivatives which have become detached from it.

3. They arise by the confluence of mesenchymal spaces, which in the mammalian embryo are frequently perivenous in formation.

After a thorough and prolonged study of an extensive series of pig embryos, the writer is forced by direct evidence to ally himself with the third position which holds that the thoracic duct has an independent origin, that it is not a product of the veins either by centrifugal growth or by the direct fusion and transformation of venous derivatives, and that its intima is a gradual differentiation from the mesenchymal reticulum. Essentially then he is in harmony with and can confirm Huntington's conclusions. Indeed so positive and convincing are the results and so perfect in their agreement among themselves that the possibility of doubting their accuracy would seem to be entirely excluded.

In further corroboration of this view is the fact that some of the individual stages of pig embryos show very clearly how the views of the venous origin of the thoracic duct sprang into existence and secured a strong foothold. During the formation of this duct the use of the injection method can produce conditions, which, disregarding all other details, would seem to corroborate the theory of its centrifugal growth. Again, in certain stages there are structures and data which would seem to furnish the necessary basis for the other view, that it arises directly from a transformed vein or its detached derivatives. But in both cases a comparative study of a sufficient number of closely graded series will prove that these appearances are due to the examination of an inadequate number of embryos, to the tyranny of one method of investigation, or to a faulty coördination of all the facts available. The appearance of centrifugal growth is plainly given by injection, but this method with all its advantages can only indicate the regions of completed channels, or the direction of their growth, and at best serve as a control by supplying negative or indirect evidence; it can never portray the actual genesis of a channel or reveal the histogenetic processes which are at work from the beginning. Furthermore, the fact that in certain stages there are venous channels which

have a course or position subsequently occupied by the thoracic duct does not imply or prove that the latter is a transformation of the former. Embryologists are learning that in order to select and understand all of the steps of embryonic changes, one embryo of each consecutive age will not suffice as was formerly the practice, but a number of embryos of the same stage are requisite. Variations do not begin with the finished organism, but they are potent throughout ontogeny from the beginning onward. Fluctuations of growth either of the whole or of parts are not infrequent, and should we base conclusions on a scanty number of embryos the chances are that they will be fragmentary or distorted. There are genetic changes of intrinsic importance, but so evanescent that we may not even catch a glimpse of them unless we have a series of embryos which approaches the ideal of complete continuity. To produce such a closely graded series may prolong the investigation and make the technic more tedious, but the end result will be more certain and will justify the labor expended. In the following descriptions of typical and consecutive stages of the thoracic duct history, and in the discussion of the data presented by them, these general considerations, as well as the various points suggested in the review of earlier investigations, will become more significant.

The fundamental genetic period of the duct, that is the time between the first appearance of its anlage and the acquisition of continuity throughout its entire extent, is of very short duration. Embryos of 18 or 19 to 23 or 24 mm., depending on individual variations, are the important ones and almost the only ones necessary for this study. In order to demonstrate more clearly, however, the relation which the development of the duct bears to the remainder of embryonic history, we must be aware of the factors and events that lead up to it or, in other words, believe it to be already potential in the period just preceding its actual inception and realization. With this supposition in mind we may artificially divide its history into three phases.

1. A veno-lymphatic phase, in which a system of provisional venous channels, or 'veno-lymphatics,' is laid down throughout the entire distance subsequently occupied by the thoracic duct.

2. A transition phase, characterized by the atrophy of these veno-lymphatics and the genesis of discontinuous lymphatic Anlagen.

3. A lymphatic phase, in which continuity is established along the whole thoracic duct Anlage and secondary growth processes bring about its completion.

Because these developmental changes proceed in a general antero-posterior direction, more than one phase may be present in the same embryo at the same time, although at different levels, but so definite is the succession of events that the above division into three phases will invariably suggest itself.

1. The veno-lymphatic phase (15-19 mm. pig embryos)

In the early embryonic history the venous plan of the thoracic region is composed of two strictly symmetrical and bilateral halves which are practically disconnected from each other except through the heart. Later by the formation of plexuses, anastomoses and fusions, certain channels acquire more of the blood current and thereby gain supremacy over others, which gradually dwindle in size and vanish and consequently give rise to the startling asymmetries of older embryonic stages and of the adult. At the time when the first of these profound transformations are initiated, a series of vessels are developed which function only temporarily in this scheme and then disappear completely. Reference is here made to the 'veno-lymphatics' which have their origin and consummation in those stages approximately between 15 and 19 mm. in length and are a part or product of the supra-cardinal or azygos system during its early transformations.

For want of a better descriptive term, the word 'veno-lymphatics' has been extensively used throughout this paper but nevertheless with some reluctance. A veno-lymphatic, as defined by Huntington and McClure in their work on the development of the jugular lymph sac in the cat, is a venous derivative which by confluence with other such channels is directly transformed into the lymphatic structure. Instead of restricting himself to this original meaning, the writer has employed this term in a somewhat different and a wider sense, as designating those temporary embryonic venous channels which occupy topographically the position of the future thoracic ducts, or other lymph ducts, but atrophy and disappear during the genetic period of these lymphatics. If this

distinction is clearly grasped all possible confusion will be avoided. There is a firm suspicion, however, that all veno-lymphatics vessels, whether apparently direct or indirect antecedents of some lymphatic, are fundamentally identical or homologous structures.

A brief account of the early history of the supracardinal or azygos system of veins will simplify the explanation of the source and character of the veno-lymphatics in the thoracic duct area and their grouping into three divisions. In 14 and 15 mm. embryos, the precardinal and jugular veins give rise, in the region of the anterior lymph sac, to a number of dorsal tributaries which are continued back to the posterior part of the body as two slender channels immediately above and parallel to the pre- and postcardinal veins. On account of their position and subsequent history, these longitudinal channels may be called the supracardinal lines. Throughout their course they are joined to the pre- and postcardinals by numerous cross-anastomoses. They also possess branches which may be described as dorsal segmental veins, because they drain the regions of the back on each side of the vertebral column and spinal cord and appear to be arranged metamerically. The disposition and fate of the supracardinal lines in a later stage (19 mm. embryo, fig. 28) can be indicated as follows: Their precardinal division (A), that segment extending between the levels of the jugular lymph sacs and the Cuvierian ducts, is complicated into a plexus, some channels of which become veno-lymphatics (6a), to be considered presently, and others are absorbed by the dorsal branches (8) of the precardinal veins. Their middle or postcardinal division (B, *12ld*, *12ls*), between the Cuvierian ducts and the anterior extent of the mesonephroi, fuses longitudinally with the postcardinal veins. The posterior division (C) furnishes the true supracardinal veins²⁰ (*12d*, *12s*),

²⁰ George S. Huntington and Charles F. W. McClure: The development of the postcava and tributaries in the domestic cat. *Am. Jour. Anat.*, vol. 6, 1907, *Abstr. Anat. Rec.*, vol. 1: "A bilateral and originally symmetrical venous channel develops dorso-medial to the primitive postcardinal vein by longitudinal anastomoses between somatic postcardinal tributaries. This secondary vein channel forms what we have termed the supracardinal system of veins. It extends from the level at which the posterior limb veins open into the postcardinals to a point cephalad where it joins that portion of the postcardinal which alone persists to form the anterior end of the adult azygos."

which proceed caudally as two large and important vessels and only very much later take a further part in the production of the asymmetrical venous plan of the trunk, especially in their transformation with the postcardinals to form the azygos system of veins; this segment may therefore be termed the supracardinal division. Since the pre- and postcardinal divisions (A, B) of the longitudinal supracardinal lines disappear or lose their independence, either by being transformed into transient venous plexuses as in the first division or by fusing entirely (12*ld*, 12*ls*) with the postcardinals as in the second division, it is evident that the dorsal segmental tributaries (8), which in the beginning spring from these lines, must shift their roots so that they arise directly from the pre- and postcardinal veins in the two divisions mentioned. In the third or posterior division (C), however, these segmental tributaries (8) continue to return their blood to the supracardinals, for the latter exist as independent venous trunks.

Because the three divisions in the transformations of the supracardinal lines coincide perfectly with, or better map out, three well-defined regions in which the history of the thoracic duct is enacted, the terms, pre-, post-, and supracardinal divisions are fully as applicable here, the events in this history occurring immediately along the pre-, post-, and supracardinal veins, respectively.

(A) *Precardinal division*. In 17 to 19 mm. embryos the anterior segment of the left supracardinal line, originally a simple longitudinal channel as in the 15 mm. embryos, has been transformed into an intricate plexus the roots of which now appear as numerous dorsal tributaries of the internal and common jugular and precardinal veins. The branches of these tributaries extend dorsad and vascularize the areas on both sides of the sympathetic nerve trunk, but in number and complexity the internal branches exceed the external ones. As will become evident later, an important distinction is potentially present between these two sets of branches, and, although the lack of differentiation at this stage would not warrant the use of specific terms, in the light of future events they may be described as precardinal venolymphatics, lying internal and mesial (6*a*, figs. 1 and 28), and precardinal segmental veins (8), functionally related to the ter-

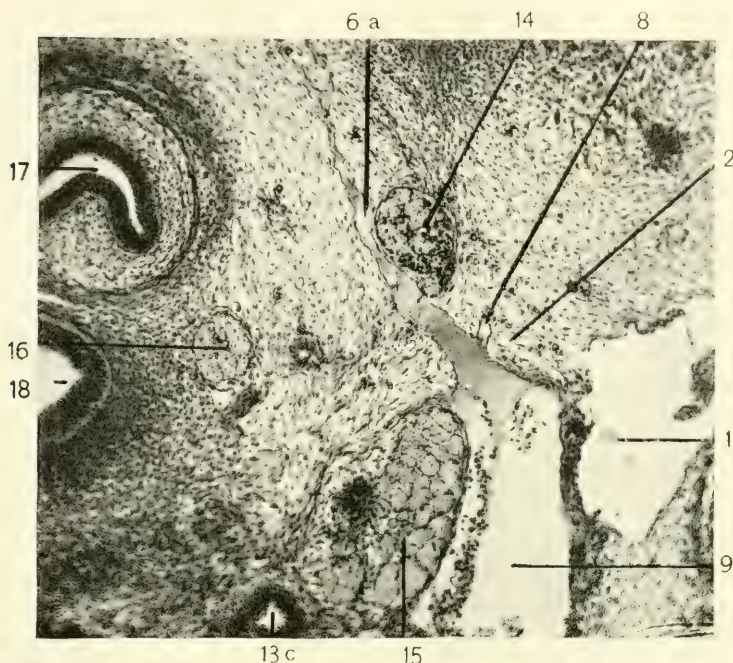


Fig. 1 Transverse section through the left jugular lymph sac region in a 19 mm. pig embryo (series 168, slide 16, section 16), $\times 100$. 1, lymph sac; 2, thoracic duct approach; 6a, precordial veno-lymphatic; 8, root of a precordial segmental tributary; 9, internal jugular vein; 13c, carotid artery; 14, sympathetic nerve trunk; 15, vagus; 16, recurrent laryngeal nerve; 17, oesophagus; 18, trachea. (Reconstruction, fig. 28.)

ritory lateral and dorsal to the sympathetic trunk. In figure 28, which represents a reconstruction of a 19 mm. embryo, these two kinds of tributaries (6a, 8) and their relations to the neighboring structures can be clearly distinguished.

Continued back from the terminals of the precordial veno-lymphatics (6a, fig. 28) is a vessel which passes obliquely over the aorta and oesophagus to enter the right postcardinal vein at the level of the Cuvierian ducts (7, fig. 28). Although it is morphologically a part of the precordial veno-lymphatics, it will often be treated separately and called the 'oblique vessel' on account of its diagonal course. The simplicity and size of this vessel varies with the individual, but it is a constant factor in all of the early

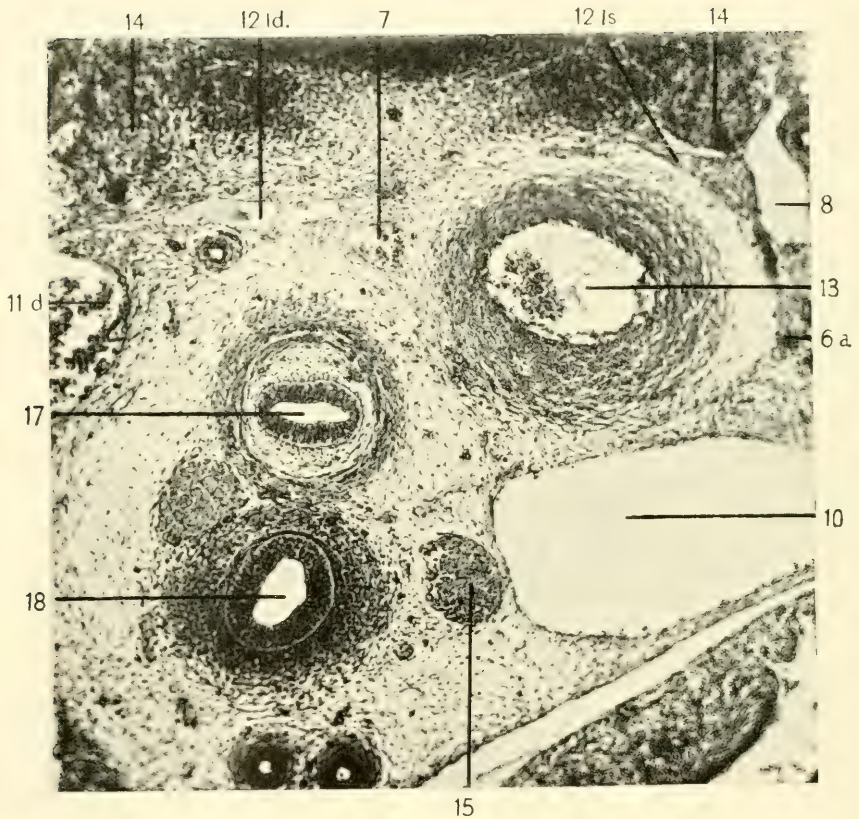


Fig. 2 Transverse section taken near the level of the left Cuvierian duct in a 17 mm. embryo (series 167, slide 15, section 22), $\times 100$. 6a and 7 (oblique vessel), precardinal veno-lymphatics located in the positions of the later right and left thoracic ducts respectively; 8, dorsal segmental vein; 10, left precardinal; 11d, right postcardinal; 12ld, 12ls, right and left supracardinal lines; 13, aorta; 14, sympathetic nerve trunk; 15, vagus; 17, oesophagus; 18, trachea.

stages of the thoracic duct history. In figure 2,²¹ it (7) is illustrated in section, taken just in front of its junction with the middle segment of the right supracardinal line, which in this embryo (17 mm.) had not yet fused throughout its entire extent with the right postcardinal.

²¹ The image of the sections being reversed by the microscope lens, the structures of the right side of the embryos are seen on the left side in the microphotographs, and vice versa. However, in the dorsal views of the reconstructions and in figure 13 the right and left sides correspond with the respective sides of the page.

(B) *Postcardinal division.* Small venous derivatives are formed by a process analogous to fenestration along the mesial border of the postcardinal (6*b*, figs. 12, 28 and 32) of each side and apparently always in the line of fusion of this vein with the middle segment of the originally independent supracardinal channel, thus suggesting their derivation from the supracardinal system also in this division. During their development consecutive ages may be distinguished among them by the amount of individuality they manifest. Some are merely little bulging irregularities in the circumference of the parent vein, others describe the first step of separation by the presence of thin strands or partitions, and still others are complete throughout a number of sections but open to the veins at one or both ends. Being in the direct axis of the precardinal veno-lymphatics and homologous with them, these venous spurs or derivatives may be called the postcardinal veno-lymphatics. Ordinarily they are exhibited more distinctly on the right side. Later they constitute longer and shorter venules (6*b*, figs. 29, 32) parallel to the postcardinal, but they are never quite independent of the latter, remaining joined to it here and there until their reduction when they break up into degenerating segments and disappear in the mesenchyme. Such a final procedure will become clearer in the consideration of the second or transition phase.

(C) *Supracardinal division.* In the region of the mesonephroi, plexuses of vessels spring from the ventral aspect of the supracardinal veins, and in some embryos they become so extensive as to encompass the aorta almost completely. For this reason and the fact that they are the equivalents of the anterior veno-lymphatics in function, they were named the supracardinal peri-aortic veno-lymphatics. A further description of them would be superfluous considering their clearness in the accompanying microphotograph and reconstruction (6*c*, figs. 3 and 32).

Caudally, at the level of the superior mesenteric artery, subsidiary channels arise from the supracardinals ventro-medially, become more and more plexiform, and approach one another from both sides to anastomose in the area dorsad of the aorta (6*c*, fig. 4). Since eventually they will be concerned in the pro-

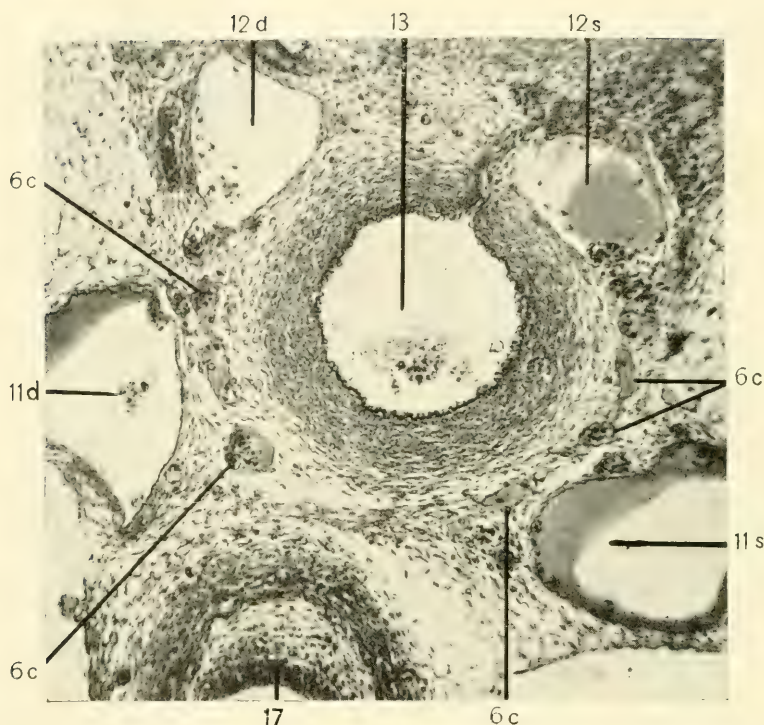


Fig. 3 Transverse section through the region just in front of the mesonephroi in a 19 mm. embryo (series 168, slide 23, section 17), $\times 150$. *6c*, supracardinal periaortic veno-lymphatics in the positions of the later right and left thoracic ducts; *11d*, *11s*, right and left postcardinals; *12d*, *12s*, right and left supracardinals; *13*, aorta; *17*, oesophagus. (Reconstructions, figs. 28 and 32.)

duction of the most posterior segment of the thoracic duct, or cisterna chyli, they may be labelled posterior supracardinal veno-lymphatics.

Situated topographically in the pathway of the future thoracic duct, all of the veno-lymphatics mentioned in the three divisions would be said to give rise to it directly, or to be transformed into it, were the second or transition phase of its history disregarded. This leads up to the suggestion that Lewis' and temporarily also McClure's multiple endothelial-lined anlagen derived from the veins along which they lie are nothing more and nothing less than the veno-lymphatics or precursors of the thoracic duct as deline-

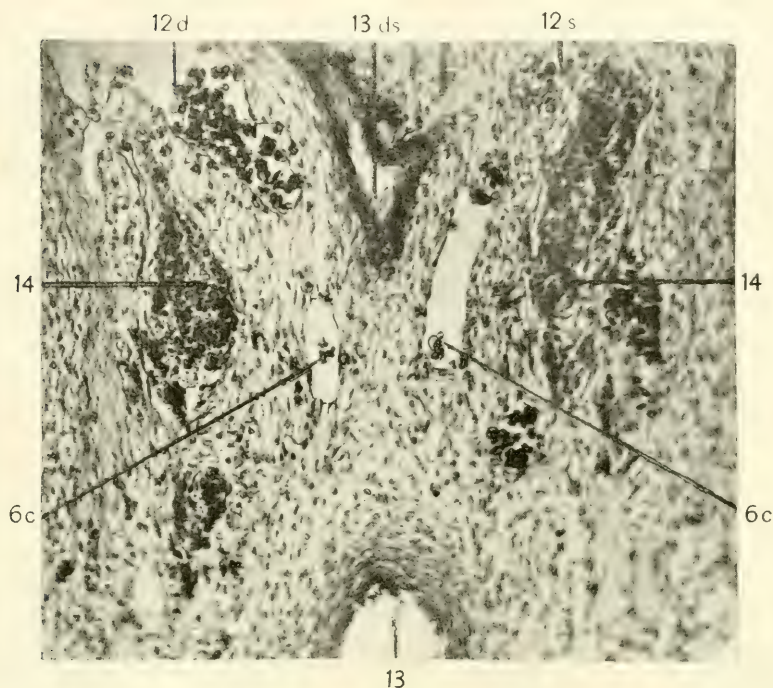


Fig. 4 Transverse section taken near the level of the superior mesenteric artery in a 20 mm. embryo (series 194, slide 40, section 16), $\times 200$. *6c*, posterior supra-cardinal veno-lymphatics, the precursors of the subsequent cisterna chyli; *12d*, *12s*, right and left supracardinals; *13*, aorta; *13ds*, dorsal segmental artery; *14*, sympathetic nerve trunks.

ated by the writer. According to the descriptions, their character and derivation in the rabbit and cat embryos accord accurately with their tendencies in the pig embryos and allow of no other conclusion than that they are homologous structures; for not only do they occupy identical positions but they also have their sources in the same venous trunks. This similarity is even more strikingly emphasized in somewhat older pig embryos where they are on the verge of evanescence and break up into isolated segments.

Until they have reached their culmination these veno-lymphatics can be followed and reconstructed with remarkable ease and accuracy. Their caliber or lumen is as uniform as a plexiform condition permits, and constrictions, irregular shrinkage, collapse,

or any other characteristic which might suggest artifacts due to the action of the preservative are nowhere in evidence. Their endothelium is tense and clear and the enclosed blood corpuscles take a clean and transparent stain.

During the veno-lymphatic phase the mesenchyme is evenly woven and fairly compact, and lymphatic anlagen, or conspicuous and discontinuous spaces, are not present throughout the entire thoracic duct area. Nor can vacuities, fissures, or rents be observed which might be ascribed to unequal fixation, or which differ in any way from the regular intercellular lacunae of the tissue reticulum.

2. The transition phase (19-22 mm. embryos)

Having arrived at the second phase of the thoracic duct history, we are confronted with the paramount point at issue, namely, the source and formation of lymphatic anlagen. A critical examination of the stages belonging to this period will disclose three facts of major importance which are impressed upon the observer firmly and constantly. In the first place the longer portion of the thoracic duct anlage arises discontinuously from mesenchymal lymphatic spaces, but it may present various aspects according to its genetic levels or to the degree of relation it bears to immediately surrounding structures, that is, it may be instituted either by extra-intimal spaces, or by spaces in the near environment of the veno-lymphatics but not in contact with them. Secondly, the wall of the entire thoracic duct is a differentiation in situ from the mesenchyme. Thirdly, the development of the thoracic duct proceeds in a general antero-posterior direction; for example, in series 194, a 20 mm. embryo, lymphatic development has made considerable progress in the anterior or precardinal division, has just been initiated in the middle or postcardinal division, and is totally lacking in the supracardinal division.

(A) *Precardinal division.* In the collection of pig embryos studied by the writer the first instances of incipient lymphatic anlagen are found in series 168 (19 mm.) along a limited extent of the precardinal veno-lymphatics as far back as the anterior half of the oblique vessel (fig. 28). Located in the path of the poten-

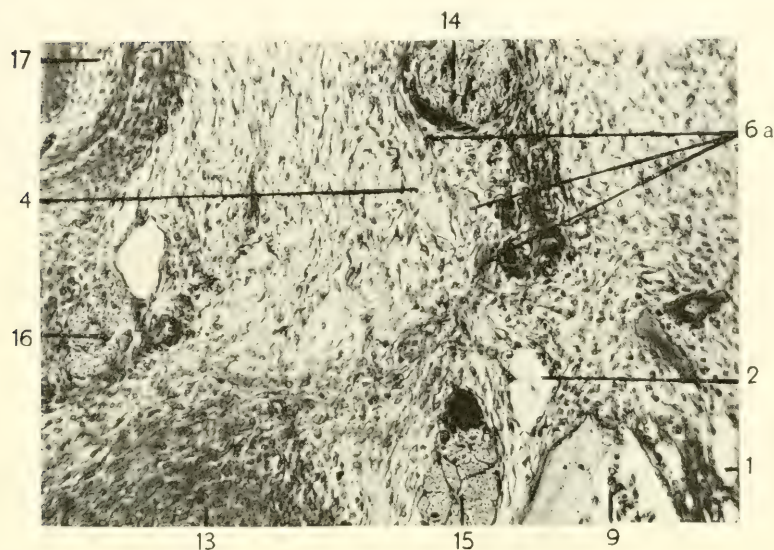


Fig. 5 Transverse section through the left lower cervical region in a 19 mm. pig embryo (series 168, slide 17, section 9), $\times 150$. 1, posterior tip of the jugular lymph sac; 2, thoracic duct approach; 4, lymphatic space formed against the intima of a precardinal veno-lymphatic, (6a); 9, internal jugular; 13, wall of the aorta; 14, sympathetic trunk; 15, vagus; 16, recurrent laryngeal nerve; 17, oesophagus. (Reconstruction, fig. 28.)

tial thoracic duct there is a distinct vacuolation of the tissue as shown in figure 6 (4), medial to a veno-lymphatic tributary (6a). Although exceedingly difficult to describe, these vacuoles or spaces are seen to stand out conspicuously, perhaps by the greater clearness of their cavities, even if their boundaries are ill-defined, and by their preponderance in size over the more regularly disposed openings of the ground substance. In a section taken further forward another lymphatic anlage occurs as a large lenticular space (4, fig. 5) against the intima of a retrogressive veno-lymphatic (6a), but it can be distinguished definitely only in two sections. In the same figure the thoracic duct approach (2) is indicated. This ends blindly but is followed shortly by large clear-cut spaces (4) which are in no way continuous with it (fig. 28). Similar and widely separated anlagen occur along the anterior or upper half of the oblique vessel (7) and immediately ventral to it.

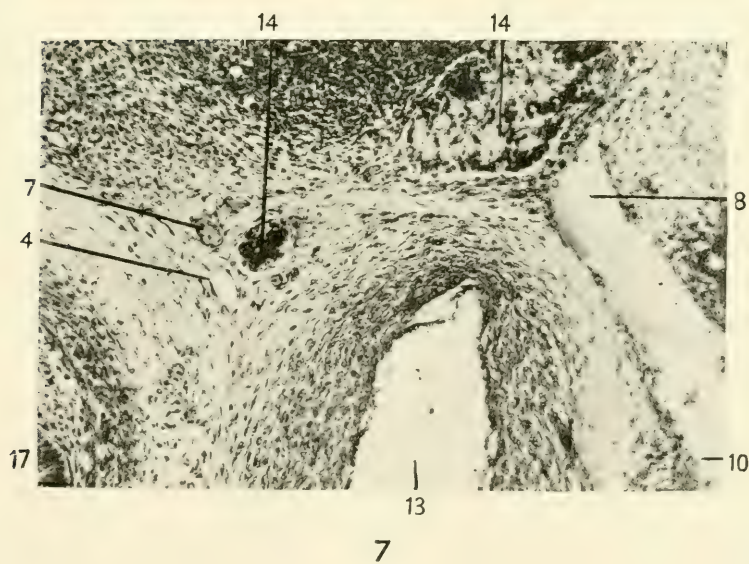
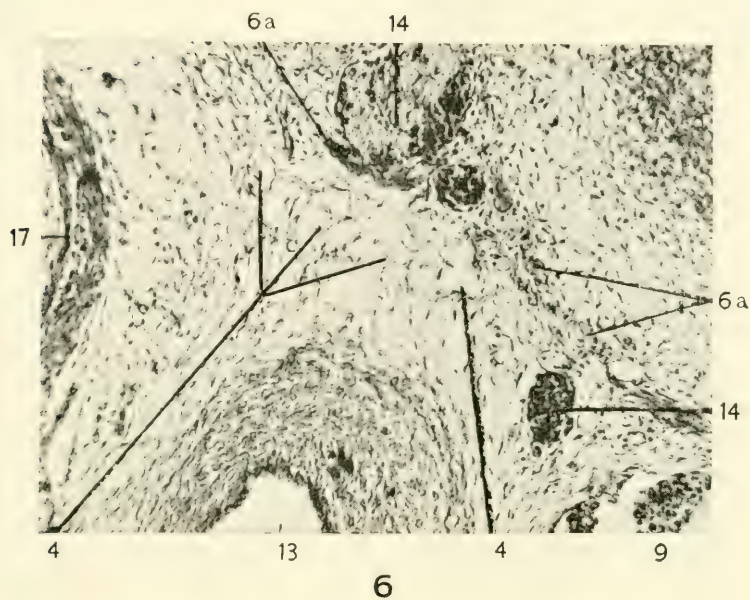
Those that were not hidden in the dorsal view of the reconstruction are illustrated in the drawing (4, fig. 28). These spaces are short capsule-shaped vesicles running through four or five sections and are quite distinct in outline, so that their extent and discontinuity can be easily determined. A transverse section of one of them is represented in figure 7 (4). The nicety with which it can be discriminated from the interstices of the surrounding mesenchyme and from the lumen of the neighboring venule which is filled with blood removes all doubt as to its reality and individuality.

On the evidence of the few microphotographs inserted here, the presence of the spaces pointed out as lymphatic anlagen can not be denied. In the embryo just described they exist only in the foremost region of the thoracic duct line; outside of this path there are no spaces which might invalidate the significance of these anlagen.

Extra-intimal replacement of evanescent venous channels, a method of lymphatic development just hinted at in figure 5 (4) finds a most convincing expression in series 194 (20 mm.), where the entire anterior precardinal veno-lymphatic plexus (6a) is being replaced by large perivenous spaces (3, fig. 29). The veno-lymphatics designated have been detached from their parent veins and thus abandoned by the systemic blood vascular circulation, and they now display successive steps towards complete collapse. The section represented in figure 8 is typical, for most of the other sections taken at random from this region offer equally decisive illustrations. Besides revealing the shriveled and discarded venous intima and its gradual disintegration in the

Fig. 6 Transverse section through the left lower cervical region in a 19 mm. pig embryo (series 168, slide 18, section 4), $\times 150$. 4, vacuolation of the mesenchyme in the formation of lymphatic spaces; 6a, precardinal veno-lymphatics, beginning to degenerate; 9, internal jugular; 13, aorta; 14, sympathetic nerve and branches; 17, oesophagus. (Reconstruction, fig. 28.)

Fig. 7 Transverse section through the left upper thoracic region in a 19 mm. pig embryo (series 168, slide 19 section 1), $\times 150$. 4, lymphatic space in the line of the future right thoracic duct; 7, oblique vessel; 8, dorsal segmental vein; 10, left precardinal; 13, aorta; 14, sympathetic nerve trunk and branch; 17, oesophagus. (Reconstruction, fig. 28.)

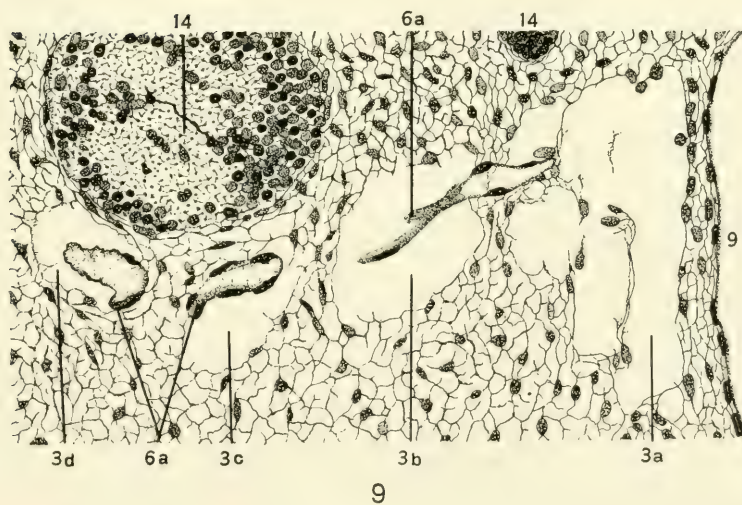
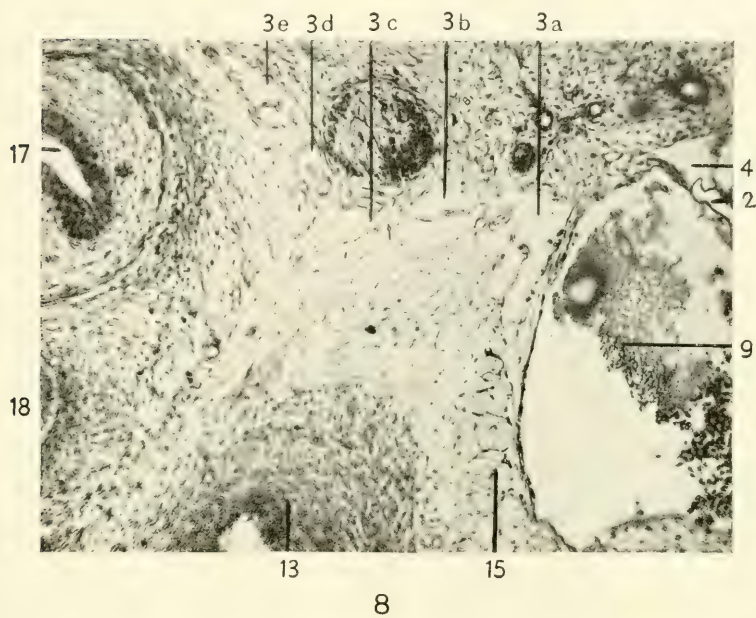


lumina of the replacing lymph vessels (*3a, b, c, d, e*), the photograph suggests the method by which the latter enlarge, as well as their mode of origin. The anlage at *3e* (fig. 8) presents an excellent initial stage in which the endothelium of the blood channel has receded from the original circumference and two small mesenchymal vacuoles have appeared one on each side of the points of weakness. On the other hand, the anlage at *3a*, the channel of the lymphatic plexus nearest to the lymph sac and internal jugular vein (*9*), is very large and irregular and has increased in size obviously by the coalescence of several closely-crowded spaces, as indicated by the extremely ragged periphery of its lumen and the remnants of tissue traversing it.

The lymphatic plexus just described is widely confluent with the jugular lymph sac (fig. 29) through the thoracic duct approach (*2*). The reader will recall that this structure, the approach, is a part of the sac and has its origin with it. In the preceding or veno-lymphatic phase it exists normally as one (fig. 28) or two, and sometimes three, short prolongations between the roots of the dorsal tributaries of the jugular vein. At the time when lymphatic spaces are appearing along the precardinal veno-lymphatics, the sharply defined venous endothelium of the approach (*2*, fig. 8) retracts from its former circumference, evidently as the result of a stagnation in its growth, and becomes surrounded by a clear and larger cavity (*4*) which is lined with ordinary unmodified mesenchymal cells, the progenitors of the

Fig. 8 Transverse section through the left lower cervical region in a 20 mm. pig embryo (series 194, slide 23, section 21), $\times 120$. *2*, thoracic duct approach, and its original venous intima replaced by a large space (*4*); *3a, b, c, d, e*, lymphatic plexus replacing as extra-intimal spaces the precardinal veno-lymphatic plexus; *9*, internal jugular; *13*, wall of the aorta; *15*, vagus; *17*, oesophagus; *18*, trachea. (Reconstruction, fig. 32.)

Fig. 9 An accurate camera lucida drawing of a highly magnified area of the section represented in figure 8, $\times 266$ (reduced from $\times 400$). *3a, b, c, d*, cross-sections of the extra-intimal lymphatic plexus replacing the anterior precardinal veno-lymphatics; the absence of any specialized endothelium in the wall of the lymphatic spaces is plainly evident; the strands of mesenchyme jutting into their cavities suggest the breaking down of contiguous spaces in the formation of the lymphatic plexus; *6a*, the collapsed venous intima of the abandoned veno-lymphatics; *9*, wall of the internal jugular; *14*, sympathetic nerve trunk and branch.



later more specialized lymphatic endothelium. Synchronously, the reorganized approach becomes confluent with the contiguous lymphatic spaces of the duct anlage by the breaking down of tissue partitions and septa between them. The vestiges of the old vascular intima may persist throughout a number of stages clinging to the wall of the new cavity, but it gradually fades and vanishes as the thoracic duct acquires more and more of its functional activity.

Histologically, all incipient lymphatic anlagen, whether they are spaces independent in position or spaces following, transforming and expanding the discarded pathways of redundant venous channels, are decidedly different from either a reticulate vein or a mature lymphatic. They lack definition and possess vague and undifferentiated outlines; for the cells of their walls are not arranged in that end-to-end fashion so characteristic of vascular endothelia. Instead, many instances were observed under strong magnification where the tissue cells in their longest diameter stand perpendicular to the periphery of the anlagen and project far into the lumen with their cytoplasmic filaments, a condition unquestionably brought about by the addition or fusion of contiguous spaces. Figure 9, which is an accurate camera lucida drawing of a highly enlarged portion of the area pictured in figure 8, should be carefully examined as depicting clearly the features here mentioned. With the most critical observation one is not able to detect differences at this stage between those cells constituting the boundaries of the lymphatic anlagen (*3a, b, c, d*) and those of the mesenchymal reticulum, either in regard to their arrangement and shape or to their staining attributes.

While there is sufficient evidence for the atrophy of venolymphatics (*6a*, fig. 9) in their elimination from the blood stream and the recession of their intima, further evidence is revealed at this stage by their reaction to the stain. Treated with heamatoxylin and orange-G, the defunct intima takes an opaque brownish color as compared with the transparency of a functional vessel. Their lumina also contain the debris of blood cells. That these conditions are not induced by poor fixation is evinced by the normal appearance of the veins in the immediate vicinity. For

example, the endothelial lining of the jugular vein (9, fig. 9) stains clearly and is sharply defined, and there are no spaces external to it.

Extra-intimal replacement occurs only among those venous channels which are immediate antecedents of lymphatics in time and place. This is attested by the fact that other veins in their atrophy are not surrounded by spaces but disappear by the gradual reduction of their caliber, or by a process of constriction cutting the channel into segments which become smaller and smaller to form dense masses or islands of cells ultimately to be lost in the mesenchyme. As an instance of such a process may be described the reduction and dismemberment of a large portion of the plexuses uniting the original supracardinal lines (25, figs. 28, 29 and 30). The writer has often noticed these temporary venous plexuses in the various stages of degeneration. In 20 mm. pig embryos, for example, such retrogressive venous channels frequently reveal constrictions at irregular intervals along their course. In later stages they begin to break up into segments, which at first, however, are still connected with one another by densely staining cell-strands, the remains undoubtedly of the endothelial walls, and in this way indicate the pathways of the originally functional vessels. The segments at the beginning possess a distinct cavity or lumen, but subsequently the cavity becomes filled up with a solid cell mass which is apparently due to a proliferation of the former endothelial or lining cells. Such cell aggregations gradually vanish in the mesenchyme perhaps by the regression of their elements to undifferentiated tissue. In other words, the cells which at one time functioned as the limiting walls of a haemal vessel, after the elimination of such a vessel from the blood channel system, lose their specialized characteristics and possibly return to the mesenchyme by assuming the qualities and functions of the ordinary tissue cell. By comparing the reconstructions illustrated in figures 28 and 35, it is seen that the venous plexuses which are so profusely developed in the 19 mm. embryo have almost entirely disappeared in an embryo of somewhat greater length. In the stained sections of the latter, however, some of the dense cellular masses are still visible here and there in

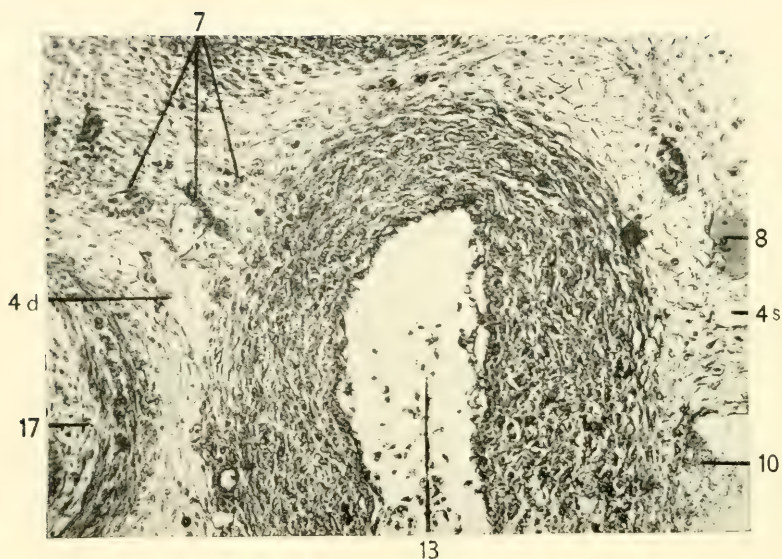


Fig. 10 Transverse section through the upper thoracic region in a 20 mm. pig. embryo (series 194, slide 26, section 21), $\times 150$. *4d*, potential lymphatic anlage in the right thoracic duct line; *4s*, lymph space in the left duct line; *7*, oblique vessel, here plexiform; *8*, dorsal segmental vein; *10*, left precardinal; *13*, aorta; *17*, oesophagus. (Reconstruction, fig. 29.)

the territory formerly traversed by the temporary venous plexuses.

In series 194 (20 mm. embryo) the atrophy of the oblique vessel (*7*) and its replacement by lymphatics has begun anteriorly, where it is supplanted to a greater or lesser degree by perivenous spaces (*4d*, fig. 29) which reflect the features of those further forward but are smaller and less conspicuous by reason of the greater simplicity of the vessel replaced. Along the second half of its course lymphatic anlagen are met with which are not formed in such intimate relations but lie ventral to and not closely in contact with it. Figure 10 illustrates a section from this region. The clear area (*4d*) subjacent to the plexiform oblique vessel (*7*) in the triangular or wedge-shaped territory between the aorta and the oesophagus unhesitatingly suggests a thoracic duct anlage in the making, as an examination of its character and a comparison with later stages seem to affirm. That this clear area is less com-

pact in texture than the tissue surrounding it is evident at a glance. Within it the cells are fewer in number, and the tissue fibrils, which appear to be more delicate than those of the mesenchymal reticulum elsewhere, enclose larger interstitial openings or tissue spaces. In longitudinal extent this potential duct-anlage, as we may call it, occurs along a considerable portion of the posterior half of the oblique vessel but varies from section to section in its definition. Often, distinct vacuoles appear suddenly in it, continue through several sections, and as suddenly disappear. Only these 'centers of space-formation,' however, can be reproduced in a model (fig. 29), the remainder of the anlage being as yet too indefinite to warrant reconstruction.

The atrophy of the oblique vessel in this specimen, series 194, serves also as a typical example of the atrophy of all temporary or redundant venous pathways, both of the veins which are the immediate antecedents of lymphatics and of those veins which are not so intimately associated with the development of a lymphatic channel. In the embryo from which figure 28 was drawn the oblique vessel (7) is still complete and continuous with the main venous trunks; in figure 29, on the other hand, it is seen to be broken up into irregular segments, some of which are replaced by extra-intimal spaces, and others gradually diminish in size and disappear in the mesenchyme adjacent to an incipient lymphatic anlage but with an appreciable amount of tissue between them. Thus it is evident that the vanishing segments of redundant venules and the growing segments of potential lymphatics may exist side by side in the same section. But now it may be asked, what distinguishes the one from the other, what basis is there for naming this one a lymphatic rudiment and that one a venous remnant, and how can both be followed to their ultimate fates without confusion? The distinction between these two vascular structures can easily be recognized beyond the possibility of a doubt. A lymphatic segment, here specifically a thoracic duct anlage, is invariably characterized by a very clear lumen and, if it is in the formative stage, by the absence of a clear-cut and specialized lining, as shown in the microphotographs already mentioned (figs. 6, 7, 8 and 10); whereas, the segments of a ven-

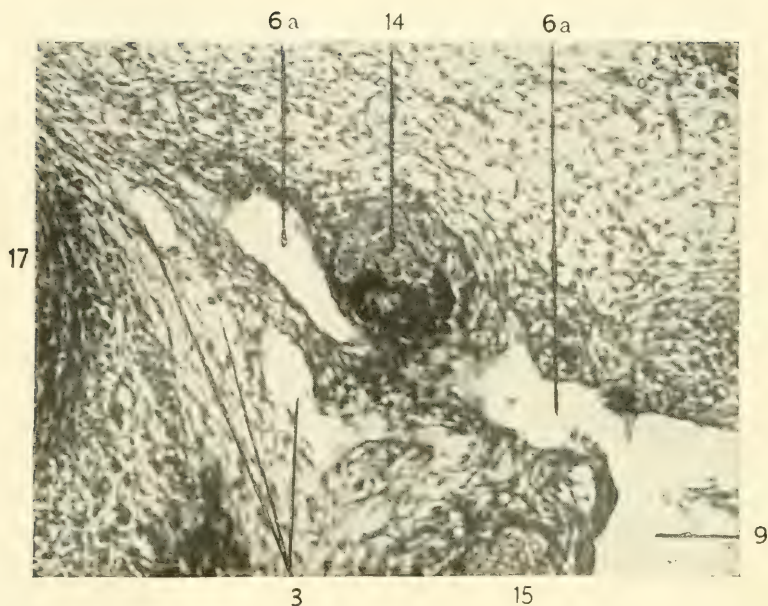


Fig. 11 Transverse section through the left lower cervical region in a 20 mm. pig embryo (series 193, slide 14, section 20), $\times 200$. This figure shows the anterior lymphatic plexus (3) of the thoracic duct not developed from extra-intimal spaces, but formed in the mesenchyme independently of the precardinal veno-lymphatics (6a); 9, internal jugular; 14, sympathetic nerve trunk; 15, vagus; 17, oesophagus. (Compare with fig. 8.)

ous channel undergoing atrophy almost constantly contain numerous blood corpuscles, or the deeply staining debris of cellular material (figs. 6, 7, 8 and 10), and without exception possess a sharply defined endothelium.

To strengthen the evidence of figures 6, 7 and 10, illustrating the formation of lymphatic spaces without the features of extra-intimal replacement, figure 11 from series 193, another 20 mm. embryo, may be introduced here as a very definite specific case to show conclusively that such replacement of venous channels is not inevitably a requisite in the genesis of lymphatics, and that its absence is quite as common as its occurrence. In this embryo the most anterior portion of the thoracic duct anlage (3) has not been modelled, so to speak, upon the anterior precardinal veno-

lymphatic plexus (6a), but has formed a plexus independently of it in a position closely parallel and medial to it. Histogenetically, however, this lymphatic plexus is the same as that of the preceding embryo (series 194, figs. 8, 29), but the redundant venous lines of which it seems a shadow picture are still quite regular, although occasional constrictions do suggest their decline and subsequent atrophy.

The foregoing descriptions of the relation between the development of lymphatic anlagen and the degeneration of the venolymphatics determine clearly that this relationship possesses only secondary significance. These two processes are necessary events in the embryonic history, and if they occur simultaneously and the abandoned venous derivatives occupy a position identical with that of the potential thoracic duct, then the anlagen of the latter will follow the path of least resistance or, better, follow a hydrostatic tendency and collect around their weakened intima and cause its collapse. If, however, these venous lines do not lie in the pathway of the duct, or if their degeneration is slightly retarded so that they are still joined to the systemic blood circulation, and are under the influence of its pressure and their intima is still tense, then the lymphatic anlagen will arise independent of any contact with them.

Fluctuations in the amount of progress attained by the right and left branches of the duct at any given moment during the critical stages of their development are not infrequent; indeed there appears to be a reciprocal action, for when one is large and long, or well represented in the number of its anlagen, the other is only scantily represented. All transition stages show these variations to a greater or lesser degree, but especially favorable examples are series 103 and 191 (21 mm. embryos), which can be regarded as complements of each other, the former being prominently dextral, and the latter sinistral in lymphatic growth. In series 103 the right limb of the duct extends as an unbroken channel far back into the postcardinal division of the thoracic region, but the left limb is just visible in its earliest rudiments as a few minute and isolated spaces. In series 191, on the contrary, we meet with a complete reversal of conditions so that the descrip-

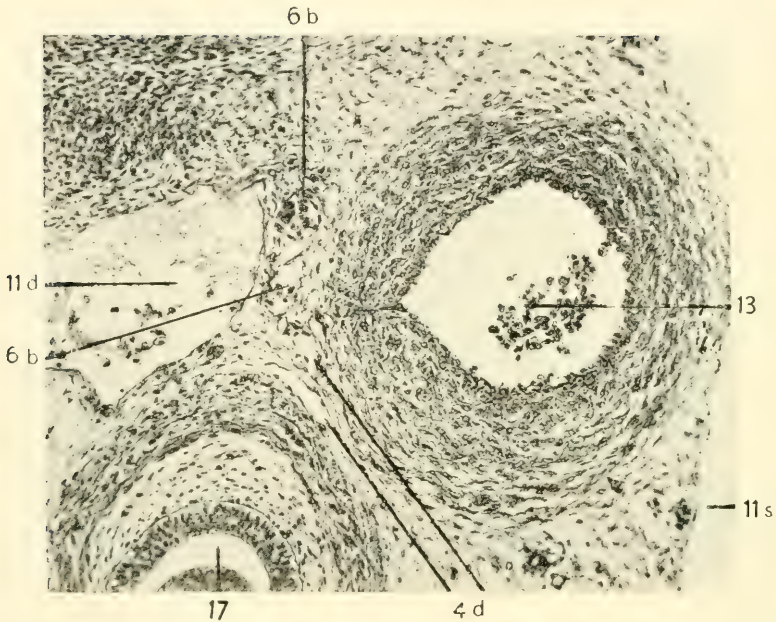


Fig. 12 Transverse section taken immediately posterior to the left Cuvierian duct in a 20 mm. pig embryo (series 194, slide 29, section 4), $\times 150$. *4d*, isolated lymphatic spaces in the right thoracic duct line; *6b*, postcardinal veno-lymphatics; *11d*, *11s*, right and left postcardinals; *13*, aorta; *17*, oesophagus. (Reconstruction, fig. 29.)

tion of the right thoracic duct limb of series 103 applies with almost equal force to the left limb of this embryo, and vice versa.

(*B*) *Postcardinal division*. In the region of the postcardinal veno-lymphatic channels the discontinuity of incipient lymphatic anlagen and their origin in situ from the mesenchyme can be plainly demonstrated. In a destined course and at intervals, though not metamerical in sequence, spaces and fissures are present in the evenly meshed tissue along the channels mentioned. For instance, they can not be mistaken in figure 12 (series 194—20 mm.), where two of them are shown as clear crevice-like spaces (*4d*) quite sharply defined, while several others are invading the environs of the veno-lymphatics (*6b*) and are beginning to enclose one of their smaller branches. In other sections this process of circumclusion has proceeded further, but in every case venous

can readily be distinguished from non-venous, the first by the presence of blood cells and heavier walls, and the second by clear cavities and more delicate walls.

In series 23a (23 mm. before fixation), an embryo somewhat older than series 194, the discontinuous lymphatic spaces of the postcardinal division are much larger and more conspicuous in the figures. Series 23a is from the Johns Hopkins University Embryological Collection and was injected and prepared by Professor Sabin. The fixation and preservation of its tissue is excellent, and the injection was successfully carried out and is as perfect as a developing lymphatic channel permits. It was sent to the Princeton Laboratory as a crucial stage in favor of the 'centrifugal growth' theory of the origin of the thoracic duct, and therefore the evidence derived from it will seem more significant perhaps than that derived from any other series described. For this reason it will here be dealt with in greater detail.²²

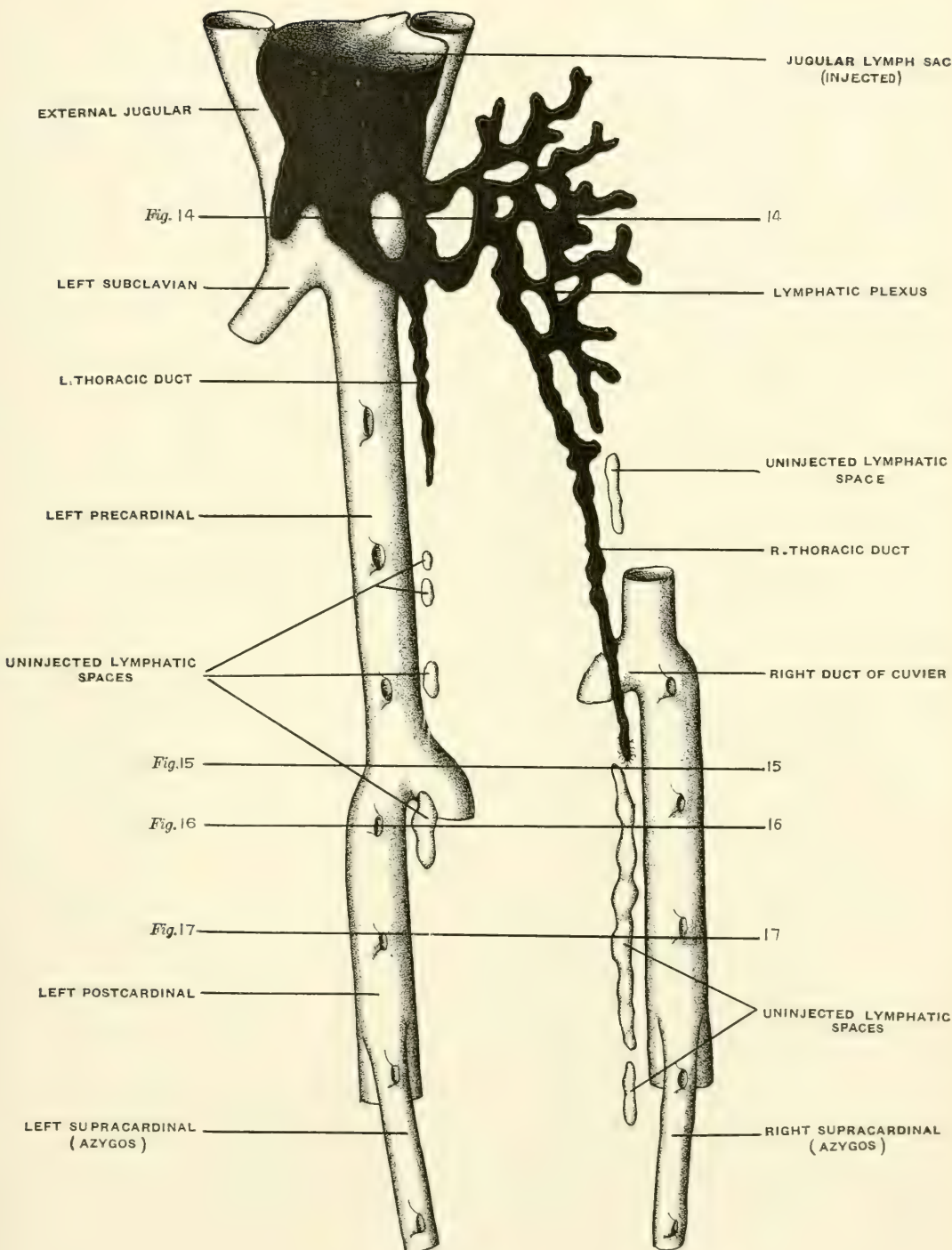
The right and left limbs of the thoracic duct anlage which are joined to the jugular lymph sac are continuous channels as far back as the points *X, X* on the drawing, figure 30, and being injected are shown in black on the diagram, figure 13. The foremost portion of the embryonic duct is in the form of a broad and extensive lymphatic plexus, a typical section of which is reproduced in figure 14 to illustrate the large size of the channels (3) and the extravasations or leakage (*Ex*) of the injection mass into the surrounding mesenchyme. To consider the right limb of the duct first, the injected vessel, or vessel confluent with the lymph sac, extends unbrokenly backward and dextrad towards the right postcardinal vein as a slender channel, subjacent to the oblique vessel (7, fig. 30) and at intervals applied to its wall, and termi-

²² The description of series 23a was presented by the writer before the last or 28th session of the American Association of Anatomists (1911) and published in the June number of the *Anatomical Record*, vol. 6, no. 5, 1912, as a part of a preliminary paper, entitled The value of the injection method in the study of lymphatic development. In this connection it is also well to call attention to Professor McClure's article, A few remarks relative to Mr. Kampmeier's paper on the value of the injection method, etc., which appeared in the same number of the *Anatomical Record*, and which is a critical analysis of some of the papers published by Professor Sabin and Dr. Eliot Clark on the development of the lymphatic system.

nates just below the level of the right Cuvierian duct at X. There is also a long and spindle-shaped space which lies lateral to it and in the pathway of a tributary of the anterior lymphatic plexus were it prolonged downward, as suggested in figures 13 and 30

Passing to the postcardinal division (B, fig. 30) of this embryo, we meet with the most decisive evidence in favor of the non-venous origin of the thoracic duct, namely, a clear case of discontinuity in its anlage than which nothing could be more conclusive. Immediately following the injected portion of the right duct (5*d*) is a long fusiform mesenchymal space (4*d*), but in no way connected with it, as exemplified by the drawings, figures 13, 30 and 31 (ventral view), and the microphotographs, figures 15, 16 and 17 which represent transverse sections taken at this level. Especially the ventral view of the reconstruction (fig. 31) illustrates the abrupt break (X) in the duct anlage, the position of the terminal portion of the injected channel (5*d*), and the independent fusiform space (4*d*) and its longitudinal extent. The injected channel ends obscurely in a 'mossy' area produced by slight extravasations, the position of which is indicated at X in figure 15 just ventral to the broad lumen of the anterior tip of the independent space (4*d*). That there is absolutely no open communication between these two segments of the duct-anlage is strikingly confirmed by both observation and experiment. In the first place the most critical examination with the high powers of the microscope was not able to detect continuity, and secondly, not a particle of the injection mass was found to have entered the cavity of the blind fusiform space (4*d*), although the pressure of the injection was sufficiently great to produce the extravasations referred to above.

Fig. 13 A simplified or schematic drawing of an accurate reconstruction of the thoracic duct region in series 23a (Johns Hopkins University Embryological Collection) represented in figure 33. The lymph sac and the injected portion of the thoracic duct anlage were drawn in black; the uninjected lymph spaces are discontinuous but are located in the axes of the injected channels and consequently in the paths of the future complete thoracic ducts. The cross lines indicate the levels at which figures 14, 15, 16 and 17 were taken.



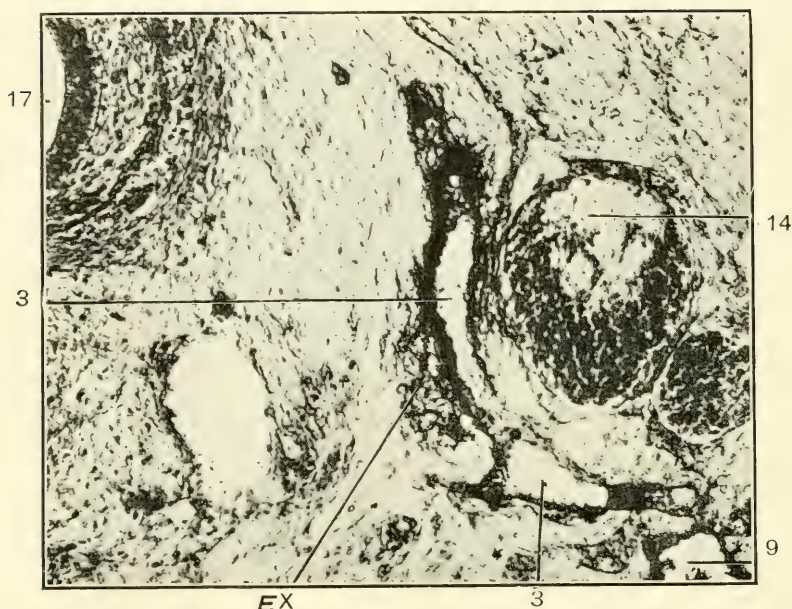


Fig. 14 Transverse section through the left lower cervical region in a 23 mm-pig embryo (series 23a, J.H.E.C., slide 21, section 16), $\times 200$. 3, anterior lymphatic plexus of the thoracic duct injected; *Ex*, extravasations of the injection substance into the surrounding mesenchyme; 9, internal jugular; 14, sympathetic nerve trunk; 17, oesophagus. (Reconstruction, fig. 30.)

The discontinuous fusiform lymphatic space (*4d*) is of considerable length, capable of being followed through thirty-seven sections (thickness of sections: 20 micra), and it is variable in diameter (figs. 31, 15, 16 and 17), at times being very broad and at other times narrow and not so sharply demarcated from the intercellular lacunae of the mesenchyme surrounding it. In form it is very irregular, and its lumen is often bridged by tissue strands of greater or lesser thickness which give to it a multilocular appearance as shown in cross-section in figures 16 and 17 (*4d*). This condition and the fact that it is bounded by ordinary mesenchymal cells supply strong proof against its venous origin. The difference between its lining and that of the neighboring venules (25) and veins is strikingly expressed even in figure 15, in which its boundary is quite regular and clear-cut but the greater delicacy

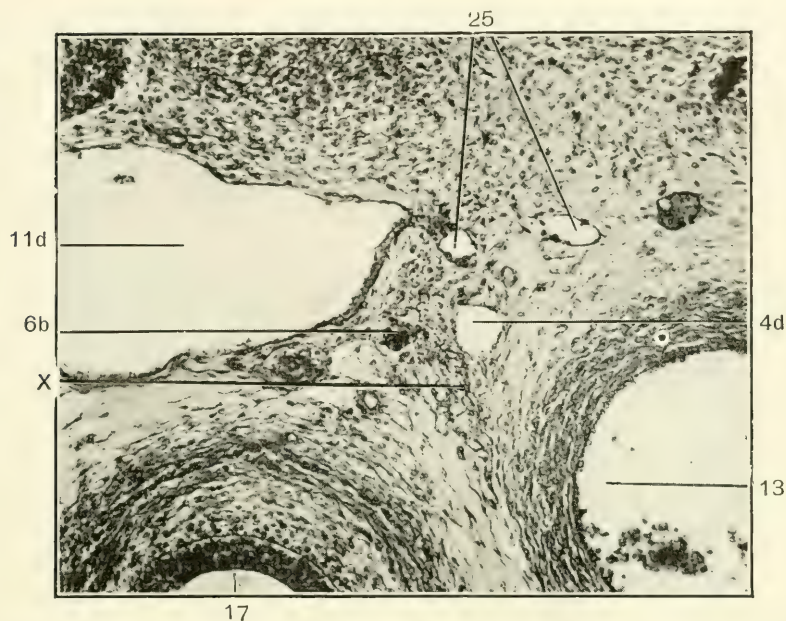


Fig. 15 Transverse section taken shortly beyond the right Cuvierian duct in a 23 mm. pig embryo (series 23a, J.H.E.C., slide 26, section 10), $\times 200$. *4d*, anterior tip of the long fusiform lymphatic space in the right thoracic duct line; *X*, position of extravasated particles from the injected portion of the right thoracic duct anlage; *6b*, postcardinal veno-lymphatic; *11d*, right postcardinal; *13*, aorta; *17*, oesophagus; *25*, venules or branches of the postcardinal. The more delicate lining of the lymphatic space as compared with that of the veins and venules can be clearly distinguished in the figure. (Reconstruction, fig. 30.)

of its wall can be distinguished without the least difficulty. Figure 17, again, illustrates the occasional circumclusion of the precardinal veno-lymphatics (*6b*) by this space and draws more plainly, perhaps, the distinction between lymphatics and venous channels, where the latter are replete with blood and possess sharply defined boundaries as compared with the often ill-defined outlines of the lymphatic space. Caudally this long space after a course which can be easily pursued through thirty-seven sections, as already stated, becomes more indistinct until it vanishes by the loss of its cavity in the confusion of the interstices of the tissue reticulum, but after a number of sections it is followed by a second space, which, though shorter and simpler (figs. 13 and 31),

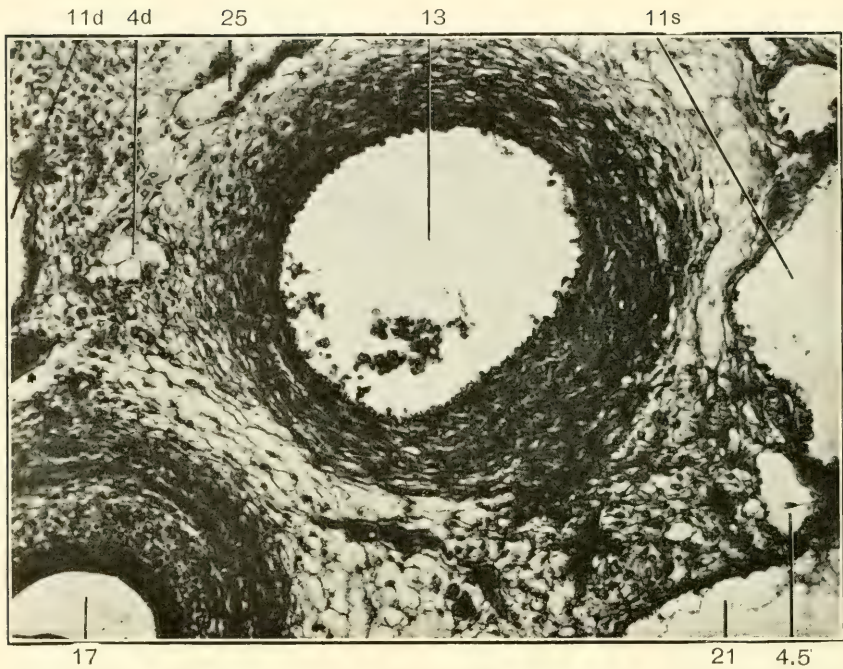


Fig. 16 Transverse section taken a few sections beyond the one represented by the preceding figure from a 23 mm. pig embryo (series 23a, J.H.E.C., slide 26, section 12), $\times 200$. *4d*, long fusiform space in the right thoracic duct line, and mesenchymal bridges traversing its lumen; *4s*, lymphatic space at the level of the left Cuvierian duct (*21*) and in the pathway of the left thoracic duct; *11d*, *11s*, right and left postcardinals; *13*, aorta; *17*, esophagus; *25*, branch of the postcardinals. (Reconstruction, fig. 30.)

exhibits the same peculiarities of character. This again is followed by tissue which is still undifferentiated but coarsely reticulate and persistently suggests the potentiality of further lymphatic anlagen. Both of the spaces described and figured are situated in the axis of the injected channel and consequently in the axis of the ultimately complete thoracic duct.

On the left side in series 23a the principle of lymphatic development is the same and is expressed fully as well as on the right side. The injected segment of the left thoracic duct limb (*5s*, figs. 30 and 13) is much shorter than that of the right, but it is slender and often it can only be traced by a 'mossy' path due to

slight extravasations. At intervals beyond the farthest extent to which the injection mass has penetrated (X, fig. 30), and located in a line destined to become the pathway of the future thoracic duct, are a number of small blind mesenchymal vacuoles (4s), the largest one of which extends through eight sections at the level of the Cuvierian duct. Being hidden by the veins in a dorsal view of the reconstruction, their positions are indicated on the drawing by dotted circles (4s, fig. 30; see also fig. 13). The conspicuous size of the lumen of the last space (4s) and the mesenchymal strand bisecting it, as illustrated in the microphotograph, figure 16, require no further comment.

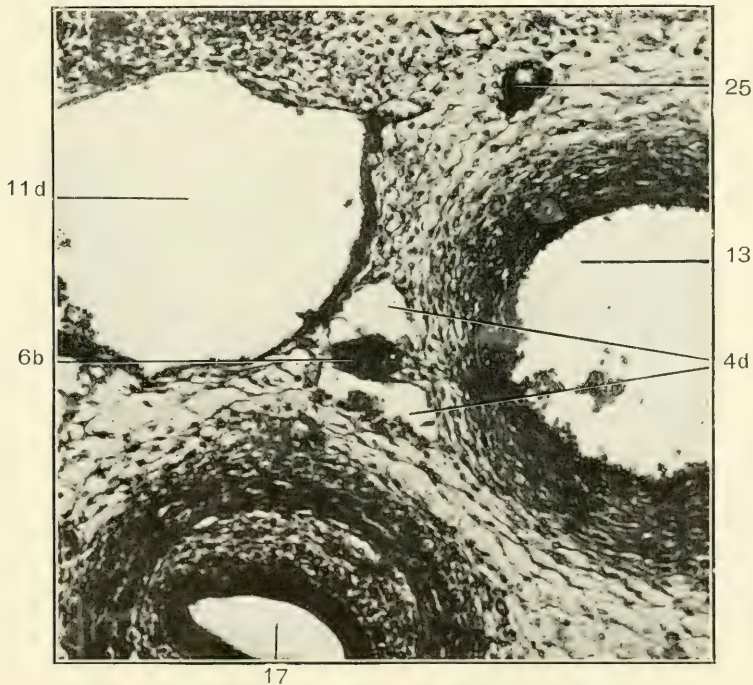


Fig. 17 Transverse section through the thoracic region in a 23 mm. pig embryo (series 23a, J.H.E.C., slide 27, section 18), $\times 200$. 4d, long fusiform lymphatic space in the right thoracic duct line, and tissue bridges traversing its lumen; 6b, postcardinal veno-lymphatic surrounded by this space; 11d, right postcardinal; 13, aorta; 17, oesophagus; 25, venule, branch of the postcardinal. (Reconstruction, fig. 30.)

In series 192 a 21.5 mm. embryo slightly older than the preceding embryo 23a, the right thoracic duct anlage (*5d*) extends as a continuous channel back to the point X, as indicated on figure 35. On comparing this with figure 30, it can be observed that the longer portion of the postcardinal division of the right anlage, which in Sabin's series 23a exists in the form of the long fusiform space described above, has established connection with the precardinal thoracic duct segment, and in this way it has increased considerably the length of the channel joined to the jugular lymph sac. Like the fusiform space of that embryo, the postcardinal thoracic duct division of series 192 is irregularly beaded or varicose, the constrictions or nodes suggesting more recent fusion between successive internodes. That this suggestion is a fair one is substantiated by the fact that toward the region of the periaortic veno-lymphatics, or beyond the point X (fig. 35) where the continuous anlage ends blindly, it is followed at intervals by a few large mesenchymal vacuoles (*4d*) between which no communication is as yet noticeable save through the indifferent tissue network.

The continuous portion of the left thoracic duct anlage in series 192 is perhaps no longer than in Sabin's series 23a, but the blind lymphatic spaces (*4s*, fig. 35) following it are far more extensive in length, especially those in the region of the left Cuvierian duct. Here there are two long spindle-shaped spaces parallel to each other, the shorter one of them being that portion of the anlage of the future mediastinal lymphatic vessel situated near the point of its subsequent junction with the other, or longer space, which represents an anlage of the left thoracic duct. In a 22 mm. embryo, a slightly older stage, all of the blind spaces of the pre- and post-cardinal divisions have become confluent to form the uninterrupted duct and its mediastinal tributary.

(C) *Supracardinal division.* In the third division of the thoracic duct area there may be recognized an anterior and a posterior half, those regions, respectively, in which the duct-anlage during its initial development is associated with the periaortic (*6c*, fig. 3) and the posterior supracardinal (*6c*, fig. 4) veno-lymphatics.

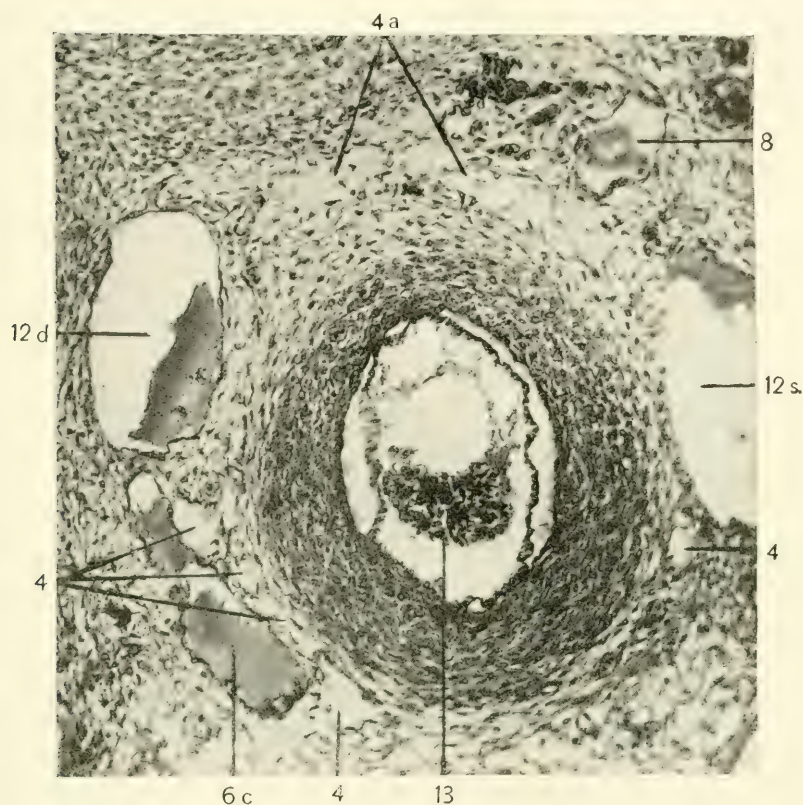


Fig. 18 Transverse section through the region of the mesonephroi in a 21 mm. pig embryo (series 103, slide 32, section 9), $\times 200$. 4, lymphatic spaces or discontinuous thoracic duct anlagen forming near or against the periaortic veno-lymphatics (6c); 4a, potential lymphatic spaces; 8, dorsal segmental vein; 12d, 12s, right and left supracoardial veins; 13, aorta.

In series 103 (21 mm.) is foreshadowed the decadence of the periaortic veno-lymphatics and the transference of supremacy to their successors. Frequently throughout their course the veno-lymphatic channels have lost their former fullness and their endothelium has been thrown into a slightly wavy and uneven contour, this being especially true of that side of the plexus facing the aorta where these areas of weakness are more abundant and accentuated. Coexistent with this condition is an incipient vacuolation

of the mesenchyme by which large and small fissures and spaces (4, fig. 18) arise irregularly and indiscriminately along the channels designated. Sometimes these spaces (4) cling closely to the receding walls of the venules (6c), and at other times they are separate with a perceptible amount of tissue intervening, but they are always discontinuous and non-venous in character. Like the more anterior lymphatic anlagen in their inception, no visible difference either of form or arrangement can be discerned between the cells which comprise their circumference and the cells of the intricate meshwork of the mesenchyme. Strands and

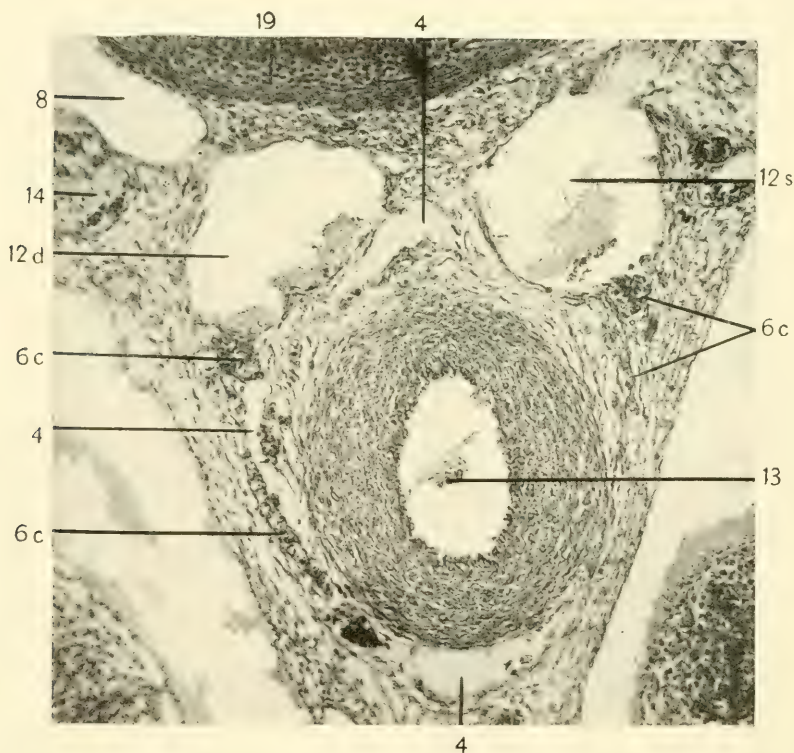


Fig. 19 Transverse section immediately in front of the mesonephroi in a 22 mm. pig embryo (series 105, slide 36, section 1), $\times 150$. 4, lymphatic spaces replacing the periaortic veno-lymphatics (6c); 8, dorsal segmental vein; 12d, 12s, right and left supracardinal veins; 13, aorta; 14, sympathetic nerve trunk; 19, embryonic vertebral column. (Reconstruction, fig. 33).

fibrils or their spur-like remnants often bridge the spaces or project into them and seem to suggest the existence of stresses and strains, as well as their direction, in the production of the anlagen by the breaking down of barriers and the fusion of interstitial spaces.

A later stage in the transformation of the periaortic region is offered by series 105, a 22 mm. embryo; but a minute account is hardly necessary considering the clearness of the appended figures which are self-explanatory and almost sufficient in themselves. Figure 19 is from a section taken just in front of the mesonephroi and is representative of the conditions active along the whole range of the periaortic plexus. The veno-lymphatics (*6c*) have lost most of their connections with the supracardinal veins and throughout the greater part of their course present a shrunken cavity filled with the deeply staining debris of blood cells. The lymphatics (*4*) which enmesh them are either broad spaces and have obliterated the venous core almost completely, or small crevices hugging one side of a vessel which has just begun to manifest degeneration. They are irregular in arrangement but they may always be distinguished by their clear lumina and unspecialized walls. A segment of the proximal portion of the supracardinal division in a 22 mm. embryo was reconstructed, a drawing of which is reproduced in figure 33, illustrating in three dimensions the conditions described.

The cisterna chyli is the outcome of a number of changes which proceed in very rapid succession and at the beginning often occur simultaneously: the detachment of the posterior supracardinal veno-lymphatics from their venous trunks; the condensation of these abandoned channels progressively toward the production of a plexiform or multilocular channel; the recession of their intima; the breaking down of broad partitions of tissue between them; the expansion of the resultant cavity by the addition of spaces from the mesenchyme; the simplification of its lumen and the acquisition of a lymphatic endothelium. In other words, a large part of the cavity of the cisterna chyli is derived from the combined cavities of preëxisting venous channels, but its wall is newly differentiated from the mesenchyme. A consideration of

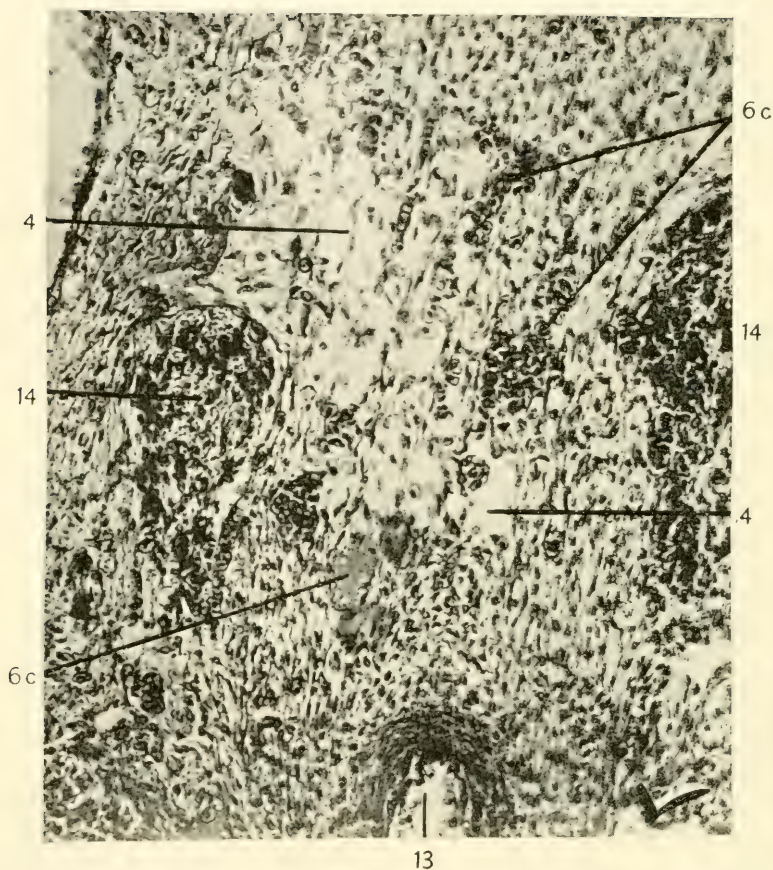


Fig. 20 Transverse section through the region of the future cisterna chyli in a 21 mm. pig embryo (series 103, slide 39, section 12), $\times 200$. 4, clear areas representing the formation of lymphatic spaces around the posterior supracardinal veno-lymphatics (6c); 13, aorta; 14, sympathetic nerve trunk.

the features revealed in 21, 22 and 23 mm. embryos will give support to these observations.

While the veno-lymphatics of the cisterna chyli region are anastomosing extensively in the median line dorsal to the aorta, they are beginning to lose their connections with the supracardinals of both sides so that they come to exist in the form of a condensed and abandoned multilocular venous plexus. Coincident with this process, the mesenchyme located between the plexiform channels

often become less compact, vesicles and fissures arise in it, and the venous intima, contiguous to these rarefied tissue-areas, retracts and breaks down; thus initiating continuity between the originally distinct channels. A section of such an incipient stage is illustrated in figure 20 which shows very clearly the vesiculated mesenchyme (4) in and amongst the veno-lymphatic plexus (6c). Typical examples of extra-intimal replacement also become very abundant and are significant as confirming the evidence already given for the reorganization of the intima whenever a lymphatic channel appropriates the pathways of redundant veins or venules. A photograph (fig. 21) of such a condition in a 22 mm. embryo describes more plainly than an extended narrative the important features in the transition period of the cisterna chyli. The compartments or loculi of the potential cisterna are traversed by distinct, delicate and devious lines which upon closer examination are found to be composed of compressed or scale-like cells, placed end to end, and to represent the discarded endothelium of the former venous derivatives (6c). This is shown when the endothelium is followed either forward or backward and it can be found occasionally lining a sharply defined cavity containing blood and then again to be pushed far into the lumen apparently by the pressure of the fluid within large mesenchymal spaces (4) on its external surface. At still other places where fusion of several parallel channels has occurred simultaneously, this evanescent venous intima is visible in cross-section as torn fibrils pendant from the irregular and frayed walls, or lying isolated in the lumen of the new or compound cavity. Besides these vestiges of the venous intima there are broader and thicker shreds of tissue, which are composed of a mass of ordinary mesenchymal cells jutting into the cavity and which indicate therefore the position of former boundaries between separate channels. Examination of the sections will also show distinctly that the outlines of the perivenous spaces are ill-defined and radically unlike those of the venous channels which they surround. In the confines of the transitional cisterna-anlage the irregular elliptical or cuboidal mesenchymal cell is the prevailing type and exists in strong contrast with the flattened and dense endothelial cell of a normal



Fig. 21 Transverse section through the cisterna chyli region in a 22mm. pig embryo (series 105, slide 42, section 8), $\times 200$. 4, anlage of the cisterna chyli showing extra-intimal replacement of the supra-cardinal veno-lymphatics (6c); 14, sympathetic nerve trunk; 13, wall of the aorta.

vein or of the rejected and defunct intima lying in the lumen of this embryonic lymphatic.

The enlargement and perfection of the developing cisterna chyli, as well as of the other segments of the thoracic duct, will be considered in the treatment of the succeeding and final phase.

3. The lymphatic phase (22–28 et seq. embryos)

At the period when lymphatic spaces are appearing around the periaortic veno-lymphatics and are growing in volume, the thoracic duct anlage has already become a continuous structure in the

pre- and postcardinal divisions. Almost concurrently the changes producing the cisterna chyli and its connection with the posterior and mesenteric lymph sacs have been active, so that the segment of the duct anlage in the territory of the periaortic vessels, at the level of the mesonephroi, is the last one to acquire continuity among the lymphatic spaces and to make the thoracic duct an unbroken tube from one extremity to the other. The reason that those portions of the duct nearest to the lymph sacs are developed first, that the vacuolation of the mesenchyme, the formation of isolated spaces, and their confluence proceeds in a general centrifugal direction is not far to seek, being probably inherent in the explanation which would make the accelerating pressure of the lymph stream towards its points of entry to the veins sufficient to account for, or at least sufficient to furnish the stimulus for, the progressive occurrence of such phenomena.

The elongation of lymphatic spaces and their fusion finally into a continuous channel, as well as the growth of their cavities in diameter, is accomplished by the same process which gave origin to them, namely, by the disintegration of tissue fibrils and the concentric addition of spaces. Figure 22 represents a typical transverse section from the precardinal division of an early lymphatic stage and illustrates very plainly how the increment in size of the thoracic duct anlage is effected. An accurate camera lucida drawing, figure 23, of a portion of the same section is also inserted here to bring out more distinctly some of the details which may be obscure in the microphotograph due to the differences in focus. Both of these figures demonstrate that the duct (5*d*) at this embryonic period enlarges by a process of growth not from within outward but from without inward, by the admission of adjacent mesenchymal spaces to its channel. The strands intersecting the lumen are therefore indicative of successive lines of fusion or a measure of its gradual growth. From the facts just stated and the exceedingly indefinite boundaries of the anlage, we should expect the absence at this time of any kind of demarcation membrane between the cavity and the interstices or lacunae of the surrounding tissue. That such is really the case is borne out by experiment. Both the veins and the thoracic duct of an

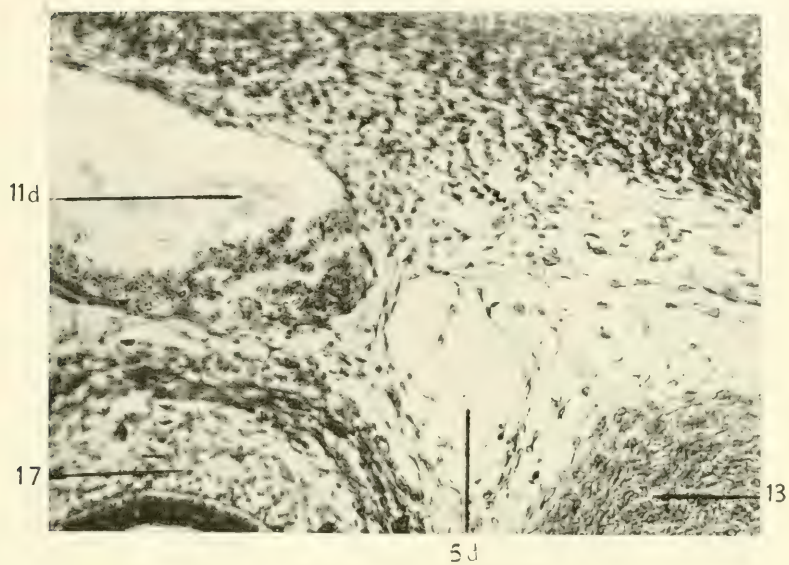
early lymphatic stage (23 mm. embryo) were injected and therefore are favorable for a comparison of their reactions to the injection mass. The veins (*11d*, *11s*, *25*, fig. 24), possessing perfect endothelial walls, did not admit of any extravasations or even of blurred outlines. In the case of the thoracic duct (*5d*), on the contrary, the injection mass (*Ex*) passed freely from the lumen into the surrounding tissue reticulum, as pictured clearly in figure 24, showing the absence of any definite wall at this early embryonic period.

Figure 25 is from the lower postcardinal division in a 26 mm. embryo and reveals essentially the same features as portrayed in figure 22 but shows even better perhaps the multilocular character of the thoracic duct anlage. The formation of spaces from the mesenchyme and their addition to the anlage is very clearly expressed on the right side in the wedge-shaped territory (*5d*) between the oesophagus (*17*) and aorta (*13*), and also on the left side (*5s*) immediately ventral to the left postcardinal vein (*11s*).

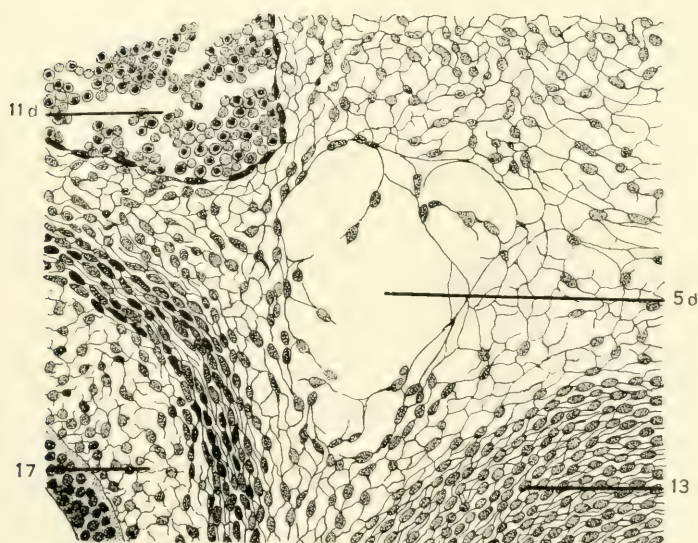
A later stage (23 mm.) in the development of the cisterna chyli (*5*) is shown in figure 26. The vestiges of the antecedent veno-lymphatics have completely disappeared, and only occasional trabeculae indicate the originally extensive tissue partitions between the early rudiments of the cisterna. What is of greater significance, however, is the ragged outline of the cavity, the absence of any specialized endothelium, and the addition of small mesenchymal spaces (*4*) to its lumen, upholding therefore in every respect the writer's contention that the cisterna chyli, concordant with the anterior divisions of the thoracic duct, is primarily and fundamentally a product of mesenchymal differentiation.

Fig. 22 Transverse section through the upper thoracic region in a 21.5 mm. pig embryo (series 192, slide 21, section 11), $\times 200$. *5d*, right thoracic duct anlage showing its enlargement by the addition of adjacent mesenchymal spaces; *11d*, right postcardinal; *13*, wall of the aorta; *17*, oesophagus; (compare with fig. 23).

Fig. 23 An accurate camera lucida drawing of a highly magnified area represented in fig. 22, $\times 266$ (reduced from $\times 400$). *5d*, right thoracic duct anlage showing its concentric growth from enlarged tissue spaces and the absence of a specialized intima; *11d*, right postcardinal vein filled with blood and possessing a well-defined endothelial lining; *13*, wall of the aorta; *17*, oesophagus.



22



23

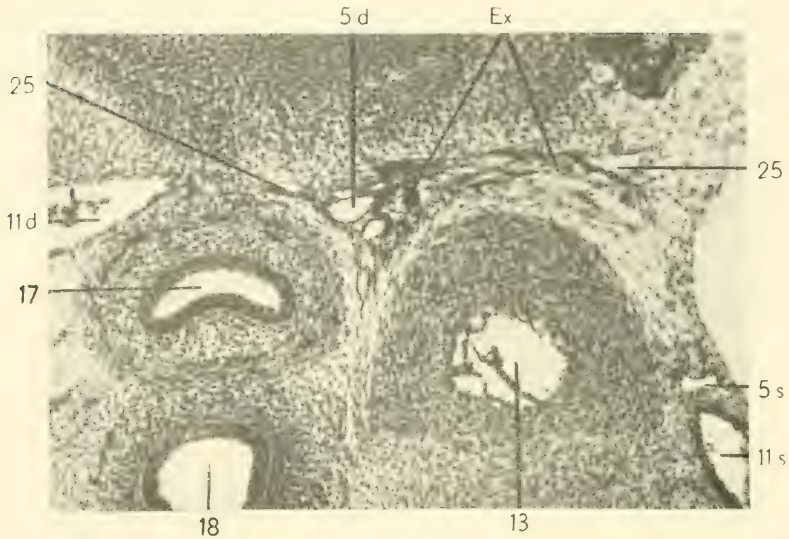


Fig. 24 Transverse section through the upper thoracic region in a 23 mm. pig embryo (injected series, slide 15, section 11), $\times 100$. *5d*, right thoracic duct anlage injected; *Ex*, extravasations of the injection mass from the right duct-anlage into the loose surrounding mesenchyme; *5s*, left thoracic duct anlage; *11d*, *11s*, right and left postcardinals injected; *13*, aorta; *17*, oesophagus; *25*, branches of the postcardinals injected.

As shown in figures 22, 23, 25 and 26, at the beginning of the lymphatic phase the thoracic duct in transverse section resembles a condensed plexus and may be said to be at the height of its complexity. For from now on there is a gradual reduction of this condition until the two limbs of the thoracic duct exist normally as single simple channels. By the breaking down of the abundant tissue bridges which had divided its channel into a labyrinth of loculi, the ducts assume more and more the appearance of unobstructed tubes. In figure 27, taken from a 26 mm. embryo, the outlines of the ducts (*5d*, *5s*) are assured, and only the vestiges of former septa and trabeculae are still visible here and there in the form of few small tissue spurs and filaments projecting into the cavities.

The most startling change, however, occurs in the precardinal division in the region of the jugular lymph sac. The reader will

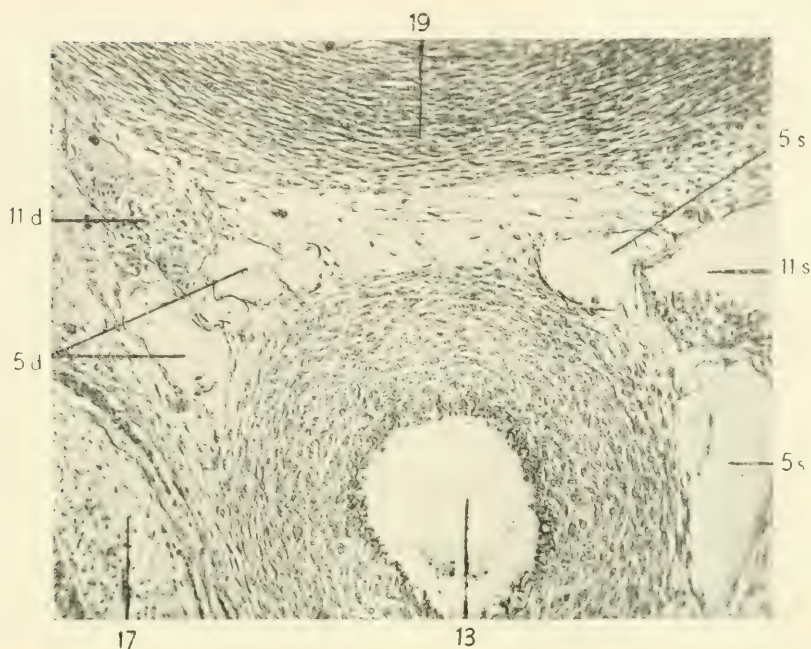


Fig. 25 Transverse section through the postcardinal region in a 26 mm. pig embryo (series 69, slide 44, section 11), $\times 150$. *5d, 5s*, right and left thoracic ducts showing the manner of their growth by the addition of spaces from the mesenchyme; *11d, 11s*, right and left postcardinals; *13*, aorta; *17*, oesophagus; *19*, embryonic vertebral column.

recall that in the later transition stages the most anterior portion of the thoracic duct anlage is characterized by an extensive and complicated lymphatic plexus, especially well developed in Sabin's series 23a (3, fig. 30). During the lymphatic phase such a plexus is completely reduced and converted into a simple channel (22–26 mm. embryos) whose fork or division into the right and left limbs of the duct becomes shifted far back of the lymph sac. Series 192 (3, fig. 35) presents an intermediate stage in which the original plexiform condition is still suggested but has been almost entirely obliterated by the transverse fusion and consequent reduction of the number of interanastomosing channels.

No matter whether the thoracic duct anlagen arise as extra-intimal spaces or entirely apart from the veno-lymphatics, dur-

ing their genesis their walls are composed of the undifferentiated mesenchymal cells (figs. 6, 9, 18, etc). We have also seen the method of concentric addition of tissue spaces by which the anlagen enlarge during the later transition and early lymphatic stages, implying thus a continual shifting of their boundaries so that an intima may be said to be temporarily established, replaced, and reorganized a number of times (figs. 22, 25, etc.) Ultimately, however, we can speak of a definite and permanent endothelium

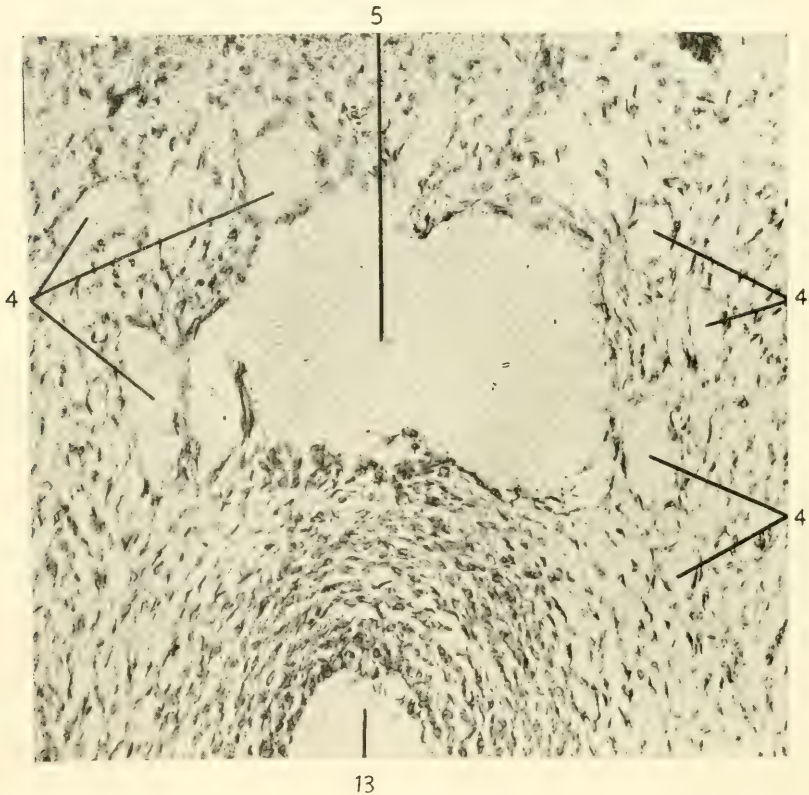


Fig. 26 Transverse section through the region of the cisterna chyli in a 23 mm. pig embryo (series 67, slide 43, section 14), $\times 150$. 5, anlage of the cisterna chyli showing the indefinite and ragged outline of its cavity, the absence of a specialized intima, and the addition of small spaces (4) from the mesenchyme; the vestiges of the former veno-lymphatics have completely disappeared; 13, aorta; 14, sympathetic nerve trunk.

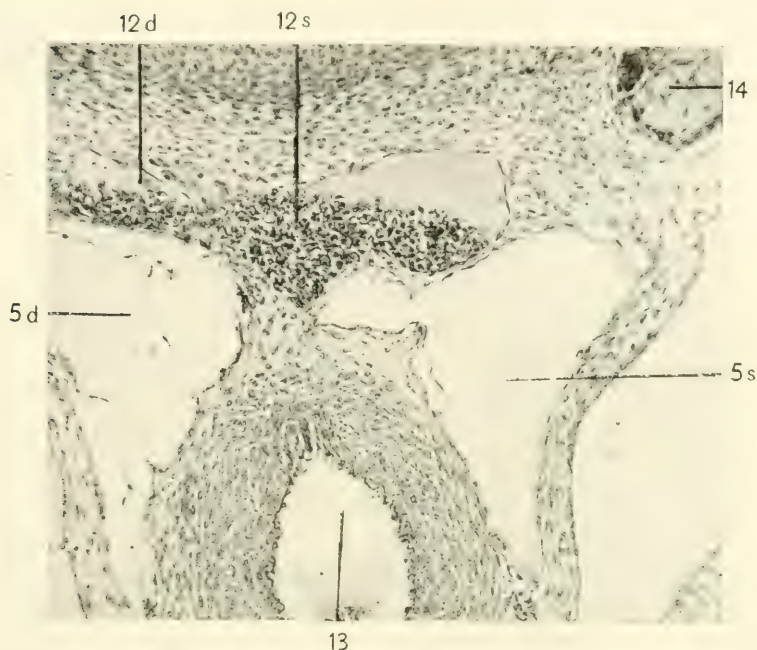


Fig. 27 Transverse section through the anterior supracardinal region in a 26 mm. pig embryo (series 69, slide 53, section 10), $\times 150$. *5d*, *5s*, right and left thoracic ducts; *12d*, *12s*, right and left supracardinal veins; *13*, aorta; *14*, sympathetic nerve trunk.

only after the thoracic duct has lost its multilocular and plexiform character, and its channel approaches more nearly to a clear-cut and simple tube (*5s*, fig. 27). The manner in which the mesenchymal cell is transformed into an endothelial cell occurs undoubtedly, as Huntington has suggested, by a mechanical adaptation to the pressure of the fluid within the lymphatic cavity. This view is entirely in harmony with the conditions observed in the extra-embryonic area of the chick²³ where the

²³ At the last session of the American Association of Anatomists, December 27, 1911, at Princeton, N. J., John E. McWhorter and Allen O. Whipple of the College of Physicians and Surgeons, Columbia University, presented a report on the development of the blastoderm of the chick *in vitro*. This report appeared as a short preliminary paper with twelve figures in the *Anatomical Record*, vol. 6, no. 3, March, 1912. These investigators, on the basis of a study of the living growing

earliest anlagen of the blood vessels arise between the mesodermal cell-strands as isolated spaces and fissures, which at first are bounded by ordinary cuboidal cells but later acquire the characteristic vascular endothelium by the modification of these cells. Being plastic, the cells lining either a haemal or a lymphatic anlage must be regarded as obeying the internal pressure of the cavity and becoming more and more flattened and endothelial-like as the pressure of the fluid or plasma increases.

Although this work deals primarily with the source of the thoracic duct, attention was not confined to it exclusively but also considered briefly two other lymph ducts, the mediastinal channel draining the mediastinum and its organs, and the right lymphatic duct, which in earlier stages of phylogenesis undoubtedly composed a part of the thoracic duct system but now ordinarily remains independent of it and receives tributaries only from the cephalic, cervical and upper thoracic regions of the right side. In development these channels repeat in all details the history of the thoracic duct, arising as isolated mesenchymal or perivenous spaces which subsequently become confluent.

After the above description of the development of the thoracic duct and a consideration of the evidence presented, attention may be directed towards several criticisms advanced recently by those investigators opposed to the view of the direct mesenchymal origin of lymphatic vessels. These opponents would dismiss as artifacts all of the 'lymphatic anlagen' described by the writer. Sabin, referring to the figures of extra-intimal replacement discovered by Huntington and McClure in their investigations on the genesis of the lymphatic system in the cat, maintains that "they are all in the center of the embryo where the fixing fluid pene-

chick embryo, brought forth conclusive evidence that the haemal channel system of the extra-embryonic area is developed from isolated spaces, which arise blindly in the undifferentiated mesenchyme and which subsequently change their shape by expansion and elongation and become confluent with other such spaces to produce the complicated blood plexuses in the area designated. The "spaces are frequently bounded by a mere line, more or less refractile in character. In others the lumen is lined with rounded or oval cells which later become fusiform and flattened.

trates last;"²⁴ and concerning all 'mesenchymal spaces,' Clark says: "They occur most often around blood vessels, and are almost certainly to be interpreted as shrinkage spaces, or spaces caused by the retraction of the mesenchyme processes made possible by slight rents produced in the preparation of the sections."²⁵ That these objections are wholly without foundation, at least in the case of the developing thoracic duct in the pig, is conclusively shown by the following observations: In the first place, the mesenchymal spaces termed lymphatic anlagen spring into existence at a definite period of embryonic history and invariably in a definite position. The embryos grouped under the veno-lymphatic phase are without an exception the younger embryos, and, although they were preserved in the same fixatives and the preparation of the sections followed the same methods as those employed for the specimens of the second phase, they do not show or even suggest instances of such anlagen. Secondly, in all of the earlier stages of the transition phase these lymphatic spaces can only be observed in the precardinal division, namely, in the territory of the most anterior segment of the thoracic duct which is formed first; in the postcardinal and supracardinal divisions the mesenchyme is still uniform, and the veno-lymphatics are functional and joined to the parent veins. In the later stages of this phase the last two segments repeat the history of the first. Thirdly, all of the extra-intimal spaces in the thoracic region of the writer's specimens occur only in connection with those venules which have been severed from their venous trunks and which lie topographically in the pathway of the potential duct. As far as the writer was able to determine, other abandoned veins existing in the same general areas and in the 'center of the body,' but not antecedent in position to the duct or to any of its tributaries, never manifest extra-intimal figures in their atrophy. All of the functional veins possess a normal and distended endothelium.

²⁴ Florence R. Sabin: A critical study of the evidence presented in several recent articles on the development of the lymphatic system. *Anat. Rec.*, vol. 5, no. 9, 1911.

²⁵ Eliot R. Clark: An examination of the methods used in the study of the development of the lymphatic system. *Anat. Rec.*, vol. 5, no. 8, 1911.

Fourthly, these mesenchymal perivascular spaces may have fused into a profuse plexus and become widely open to the jugular lymph sac as in the case of series 194 (fig. 29), but they, as yet, do not show a well-defined or specialized wall (figs. 8 and 9). In the fifth place, all of the discontinuous mesenchymal spaces follow one another in a succession practically undeviating which represents an outline or fragmentary picture of the future duct. Outside of this line there are no lymphatic spaces. Sixthly, in the third or lymphatic phase, when continuity of the duct and its branches has been established, no perivenous or other isolated vacuities can be discovered. If the discontinuous lymphatic anlagen were artifacts we should expect to find the largest number of them in this last phase because the diameter and the bulk of the embryos are greater, and therefore the longer time required for the fixing fluid to penetrate to their centers would make possible greater unevenness of fixation and consequently greater shrinkage.

Because the elongation of the thoracic duct is effected by a progressive summation or centripetal addition of large mesenchymal spaces to that part of the anlage already confluent with the lymph sacs, the injection of successive transition stages up to the time when continuity has been acquired throughout its entire course will show a gradual increase in the length to which the injection mass has penetrated; but the study of serial sections will also reveal anlagen which lie beyond the farthest point of the injection and are inaccessible to it on account of their discontinuity, or because they have not as yet become confluent with the anlage into which the injecta were introduced.

This leads to a second contention of Sabin, namely, that the study of serial sections alone is inadequate, and that continuity of the apparently discontinuous lymphatic anlagen can be demonstrated by complete injection. A more radical refutation of this argument than that furnished by her own series 23a is scarcely possible. The abrupt break between the precardinal injected segment of the right thoracic duct anlage in this embryo and the postcardinal uninjected segment (fig. 31) bears out in a striking manner the evidence derived from the writer's series. Notwith-

standing the inability of the eye to discover a connection between these two segments with the aid of high magnifications, it might be urged by those prejudiced that the injection may have been only a partial one. But this objection becomes groundless when the reader recalls that the pressure of the injecting fluid was of sufficient force to produce extravasations, which, as Clark maintains, signify an excess of pressure in filling the cavity completely; for he says, "With too great pressure there is produced a mossy appearance around the capillary (lymph), as has been pointed out by Hoyer, due evidently to forcing the injection mass through the lymphatic wall." If an opening had been present between these two anlagen the injecting substance would certainly have obeyed the direction of least resistance and passed into the second one. Nor is the objection valid which would exclude this large blind fusiform space from taking any significant part in the production of the thoracic duct; for not only is the distinct character and position of this space contrary to such a view but also the fact that the left side discloses similar spaces located in the identical line of the future left duct. Somewhat later embryonic stages make these observations conclusive; for example, in series 192 the post-cardinal segment of the right duct duplicates or agrees in all of its features with that of series 23a, except for its continuity with the anterior or precardinal segment (figs. 30, 31 and 35) and consequently with the jugular lymph sac. Moreover, during the progress of his investigation the writer has tentatively assumed the possibility of a centrifugal growing of thoracic duct buds through the large mesenchymal spindle spaces situated only in the thoracic duct pathway, and he has searched for such hypothetical sprouts but has not succeeded in finding a trace of evidence in their favor.

Sabin's and Clark's contention that discontinuities in a lymphatic channel are due to artifacts, resulting during fixation from the unequal shrinkage here and there of its caliber, is easily controverted by the observed facts. In the case of the developing thoracic duct such discontinuities only occur in the stages of the transition phase, in those embryos measuring approximately between 20 and 23 mm. The discontinuous segments or anlagen begin as minute mesenchymal vacuoles which gradually enlarge

and elongate with the increasing age of these embryos; in other words, in a 22 mm. embryo the blind segments of the duct anlage will be much longer and more conspicuous than in a 20 mm. embryo for instance. Further, there is a positive regularity in the progressive reduction of the number of these blind lymphatic anlagen in a general antero-posterior direction by their addition to the continuous anlage, which, as a consequence, gradually becomes elongated. Were these lymphatic spaces artifacts, or segments cut off from a continuous channel by shrinkage, then the determinate sequence of genetic changes pointed out in the descriptions of the individual stages could not exist, and we should find them in slightly older embryos or in those portions of the duct-anlage definitely known to be complete, for the same methods of technic should produce similar effects. The embryonic thoracic ducts when fully formed and indeed all lymphatic vessels possess a varicose channel constricted and dilated alternately into irregular nodes and internodes. Such a condition, however, is not brought about by fixation but is a characteristic peculiar to a lymph vessel and obviously harks back to the period when it was composed of a varying number of irregular fusiform or oblong mesenchymal spaces succeeding one another with distinct interruptions. Accordingly, the nodes or constrictions of a thoracic duct just completed would indicate the areas of final fusion between consecutive anlagen.

It should be emphasized here that Sabin and Clark base their criticisms chiefly upon the latter's investigations on the development of the lymphatic capillaries in the tail fin of the larval frog. The fallibility of their argument becomes therefore further evident when we find them comparing the reaction to the fixatives of these terminal lymphatics with that of other lymph channels, especially the larger ducts and trunks; for, although the principle of development probably is the same in both cases, the details of their behavior during the preparation of the sections may be quite different. It would be just as logical to describe a large systemic artery or vein entirely in terms of their terminal arterioles or venules. The writer will not deny that careless or imperfect fixation may cause the delicate capillaries of the fin of a tadpole

to shrink into seemingly isolated segments so that they can be pursued only with great difficulty, as described by Clark, but he does deny, supported by the decisive evidence of the injected series 23a and reinforced by all of the transition stages, that the discontinuous anlagen observed by him and invariably found to be concomitants in the formation of a large lymphatic trunk like the thoracic duct are artifacts, produced by the preserving or fixing reagents.

V. RÉSUMÉ OF OBSERVATIONS AND CONCLUSIONS

1. Derived from the supracardinal or azygos system of veins, a series of venous channels, called veno-lymphatics, are formed in the pathway finally occupied by the thoracic duct, and at the culmination of their development they exist as plexuses of vessels abundantly connected with the parent veins.

2. The actual genesis of the thoracic duct is initiated by the appearance of blind mesenchymal lymphatic spaces either around or not immediately in contact with the venous derivatives, or veno-lymphatics, which become detached from their venous trunks and break up into degenerating segments. The lymphatic spaces or anlagen arise by the local disintegration of the fibrils of the tissue reticulum and the fusion of the interstitial lacunae, and they enlarge and elongate in a similar manner. If they are of the nature of extra-intimal spaces the endothelium of the evanescent abandoned veno-lymphatics, which they replace, collapses as the result perhaps of the increasing influence of the lymph pressure on its external surface after its release from the blood pressure. During their inception and growth the walls of the discontinuous thoracic duct anlagen are composed of the ordinary unmodified mesenchymal cells. That such lymphatic anlagen are not artifacts is shown by their definite position and period of formation and the determinate sequence from their first appearance as mesenchymal vacuoles, through the phase of their growth and elongation, to their final fusion into a continuous channel. In the production of the most posterior portion of the thoracic duct, or cisterna chyli, veno-lymphatic channels by fusion with

one another give rise to the larger part, perhaps, of its cavity; but at the same time their endothelium recedes and degenerates, and the cisterna-anlage increases in size by the addition of spaces from the mesenchyme, so that, like the more anterior segments of the thoracic duct anlage, it is bounded by ordinary embryonic tissue cells during this early developmental period.

3. The elongation and final continuity of the thoracic duct anlage is effected by the progressive confluence of discontinuous fusiform lymphatic spaces in a general centrifugal direction, probably determined by the impulse of the lymph flow towards the radiation centers or lymph sacs. Injected specimens of the early lymphatic stages certify the reality of blind uninjectible anlagen beyond the farthest points to which the injecta have penetrated, demonstrating that discontinuities in a developing lymphatic channel are not 'appearances' found only by the study of uninjected embryos. Not a shadow of evidence was discovered in favor of the theory which maintains the centrifugal growth of the duct by budding from the lymph sacs or the derivation of the lymphatic endothelium from the veins. During the period of its initial growth the thoracic duct increases in diameter by the concentric addition of enlarged and immediately surrounding tissue spaces to its lumen. The intima of the thoracic duct is a differentiation in situ of mesenchymal cells as an adaptation probably to the pressure of the lymph flow within the cavity.

PLATES

PLATE 1

EXPLANATION OF FIGURE

28 Reconstruction of the vascular channels of the lower cervical and thoracic regions in a 19 mm. pig embryo (series 168, slides 16-24 inclusive), $\times 50$. Dorsal view. Arrows indicate the levels at which the microphotographs were taken. Cross lines, not labelled, indicate the extent of the divisions, A, B, and C.

- | | |
|--------------------------------------------------------------------------------|------------------------------------------------------|
| A, precardinal division | 12ls, left supracardinal line, plexiform |
| B, postcardinal division | 13, aorta |
| C, supracardinal division | 13a, aortic arch |
| 1, left jugular lymph sac | 13c, left carotid artery |
| 2, thoracic duct approach | 13ds, dorsal segmental arteries |
| 4, lymphatic spaces, incipient thoracic duct Anlagen | 13s, left subclavian artery and branches |
| 6a, precardinal veno-lymphatics | 14, left sympathetic nerve trunk |
| 6b, postcardinal veno-lymphatics | 15, vagus |
| 6c, supracardinal veno-lymphatics | 16, recurrent laryngeal nerve, and accompanying vein |
| 7, oblique vessel | 17, oesophagus |
| 8, dorsal segmental veins of the pre-, post-, and supracardinals, respectively | 20, mesonephroi |
| 9, internal jugular vein | 21, left Cuvierian duct |
| 10, left precardinal vein | 22, left subclavian vein |
| 11d, 11s, right and left postcardinal veins | 23, cephalic vein |
| 12d, 12s, right and left supracardinal veins | 24, external jugular vein |
| 12ld, right supracardinal line beginning to fuse with the right postcardinal | 25, venous plexus between supracardinal lines |
| | 26, subclavian approach of jugular lymph sac |
| | N5, fifth spinal nerve |

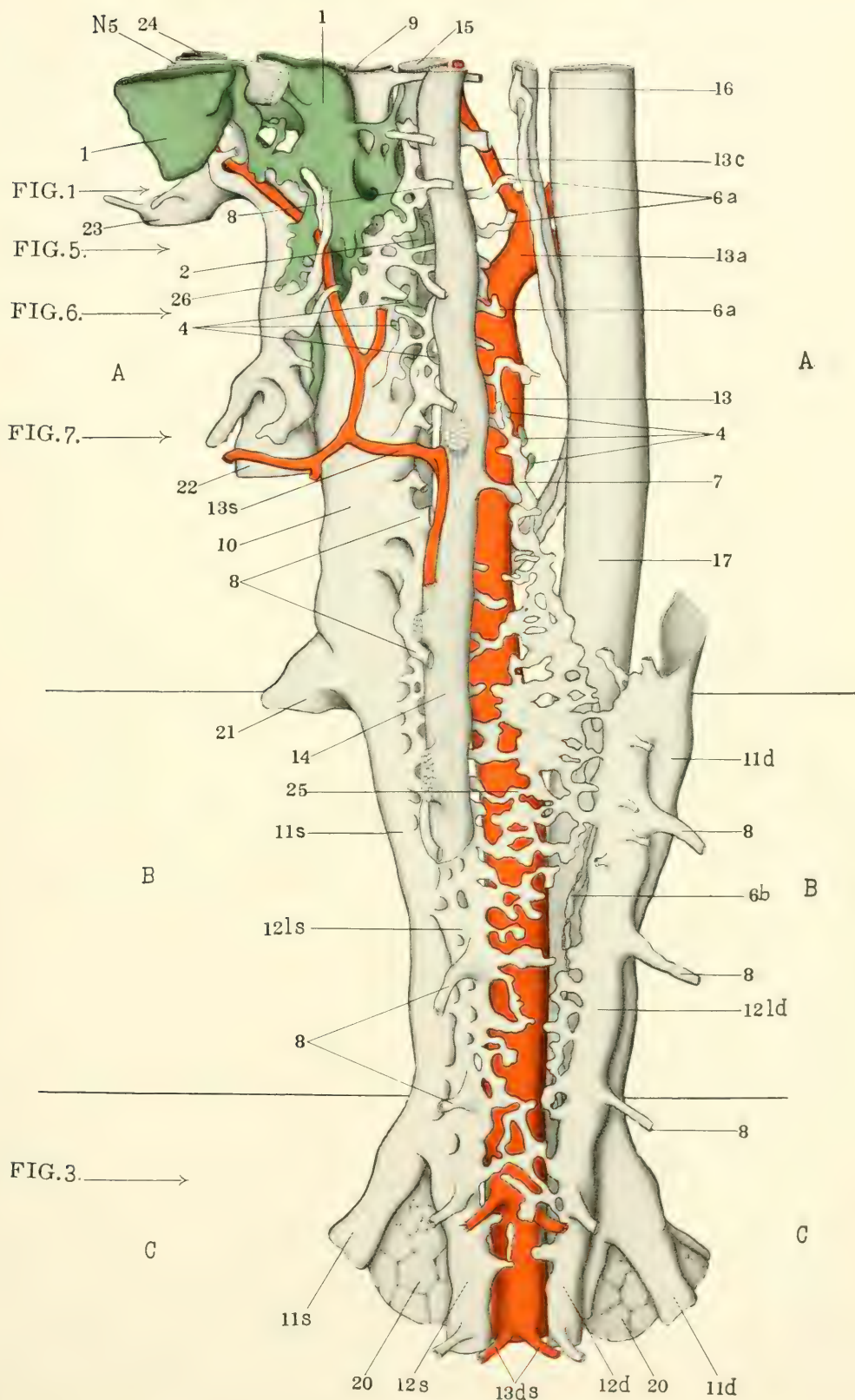


PLATE 2

EXPLANATION OF FIGURE

29 Reconstruction of the vascular channels of the lower cervical and thoracic regions in a 20 mm. pig embryo (series 194, slides 22-31 inclusive), $\times 50$. Sinistro-dorsal view.

- | | |
|------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------|
| A, precardinal division | <i>12d, 12s</i> , right and left supracardinal veins |
| B, postcardinal division | <i>12ls</i> , left supracardinal line; right line has completely fused with the right postcardinal |
| C, supracardinal division | <i>13</i> , aorta |
| <i>1</i> , left jugular lymph sac | <i>13b</i> , ductus arteriosus Botalli |
| <i>2</i> , thoracic duct approach | <i>13s</i> , left subclavian artery and branches |
| <i>3</i> , anterior lymphatic plexus or subsequent common trunk of the right and left thoracic ducts replacing the precardinal veno-lymphatics | <i>14</i> , left sympathetic nerve trunk |
| <i>4d</i> , lymphatic spaces in the right thoracic duct line replacing the oblique vessel | <i>15</i> , vagus |
| <i>4s</i> , lymphatic spaces in the left thoracic duct line | <i>16</i> , recurrent laryngeal nerve |
| <i>6a</i> , precardinal veno-lymphatics | <i>17</i> , oesophagus |
| <i>7</i> , oblique vessel, degenerating and breaking up into segments | <i>21</i> , left Cuvierian duct |
| <i>6b</i> , postcardinal veno-lymphatics | <i>22</i> , left subclavian vein |
| <i>6c</i> , supracardinal veno-lymphatics | <i>23</i> , cephalic vein |
| <i>8</i> , dorsal segmental veins of the pre-, post-, and supracardinals | <i>24</i> , external jugular vein |
| <i>9</i> , internal jugular vein | <i>25</i> , degenerating remnants of the former venous plexus between the supracardinal lines. |
| <i>10</i> , left precardinal vein | <i>26</i> , subclavian approach of the jugular lymph sac |
| <i>12d, 11s</i> , right and left postcardinal veins | <i>N5</i> , fifth spinal nerve |

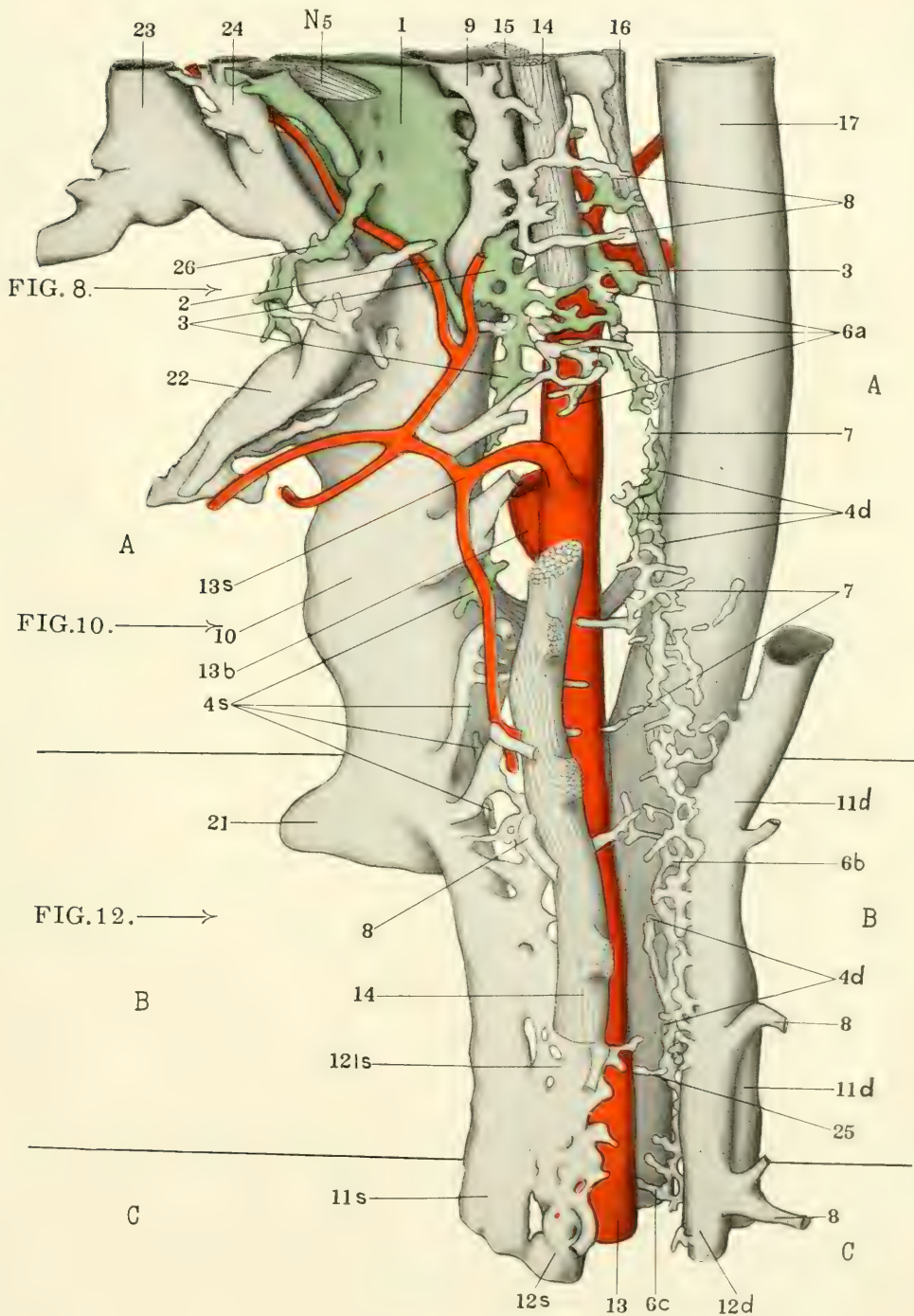


PLATE 3

EXPLANATION OF FIGURE

30 Reconstruction of the lower cervical and thoracic regions in a 23 mm. pig embryo (injected series 23a, Johns Hopkins University Embryological Collection, slides 21-30 inclusive), $\times 50$. Dorsal view, slightly from the left.

- | | |
|-----------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------|
| A, precardinal division | 10, left precardinal vein |
| B, postcardinal division | 11 <i>d</i> , 11 <i>s</i> , right and left postcardinal veins |
| C, supracardinal division | 12 <i>ls</i> , left supracardinal line, plexiform; the right line has fused with the right postcardinal vein |
| 1, left jugular lymph sac | 12 <i>d</i> , 12 <i>s</i> , right and left supracardinal veins |
| 3, anterior lymphatic plexus or subsequent common trunk of the right and left thoracic ducts. | 13, aorta |
| 5 <i>d</i> , 5 <i>s</i> , continuous and injected portions of the right and left thoracic duct anlagen | 13 <i>s</i> left subelavian artery and branches |
| X, X, extent of the continuity of 5 <i>d</i> and 5 <i>s</i> , and the farthest points to which the injection mass has penetrated | 14, left sympathetic nerve trunk |
| 4 <i>d</i> , long and short lymphatic spaces in the axis of the injected anlage and in the path of the future right thoracic duct | 15, vagus |
| 4 <i>s</i> , lymphatic spaces in the left thoracic duct line hidden by the veins, but indicated by the dotted circles | 16, recurrent laryngeal nerve, and accompanying vein |
| 7, oblique vessel, degenerating anteriorly | 17, oesophagus |
| 8, dorsal segmental veins of the pre-, post, and supracardinals | 20, anterior tip of left mesonophros |
| 9, internal jugular vein | 21, left Cuvierian duct |
| | 22, left subelavian vein |
| | 23, cephalic vein |
| | 24, external jugular vein |
| | 25, degenerating segments of the former extensive plexus between the supracardinal lines |
| | 26, subelavian approach of the jugular lymph sac |

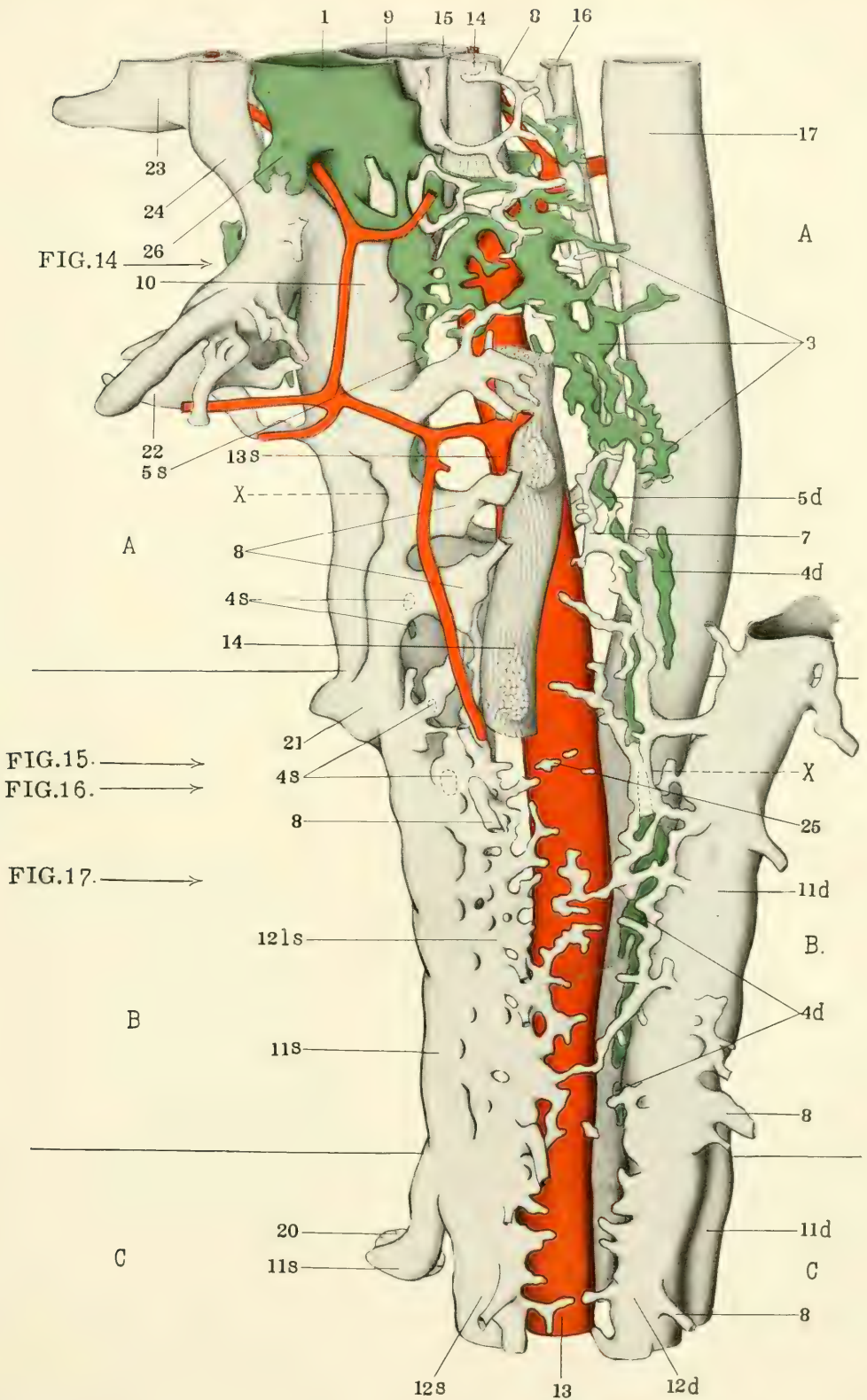


PLATE 4

EXPLANATION OF FIGURE

31 Ventral view of the lower half and right side of the reconstruction represented in figure 30 (23 mm. pig. embryo, series 23a, J.H.E.C., from section 12, slide 25 to slide 30 inclusive), $\times 50$.

- | | |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <p><i>5d</i>, extremity of the injected portion of the right thoracic duct</p> <p>X, blind end of <i>5d</i> and the farthest extent to which the injection mass has penetrated</p> <p><i>4d</i>, discontinuous and uninjected lymphatic spaces in the right thoracic duct line</p> | <p><i>6b</i>, postcardinal veno-lymphatics</p> <p>7, posterior portion of the oblique vessel and its junction with the postcardinal</p> <p>8, dorsal segmental veins</p> <p><i>11d</i>, right postcardinal vein</p> <p><i>12d</i>, right supracardinal vein</p> <p><i>13</i>, aorta</p> |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

32 Sinistro-ventral view of the lower third of the reconstruction shown in figure 31 (19 mm. pig embryo, series 168, slides 22-24 inclusive), $\times 50$.

- | | |
|------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------|
| <p><i>6c</i>, periaortic supracardinal veno-lymphatics</p> <p><i>11s</i>, left postcardinal vein</p> | <p><i>12s</i>, left supracardinal vein</p> <p><i>20</i>, right mesonephros</p> |
|------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------|

33 Reconstruction of a segment of the anterior supracardinal division or region of the periaortic veno-lymphatics in a 22 mm. pig embryo (series 105, slides 34-36 inclusive), $\times 50$. Dextro-ventral view.

4d, *4s*, lymphatic spaces replacing the degenerating veno-lymphatics (*6c*). Other explanations the same as above.

34 Reconstruction of the same region, represented in the preceding figure, in a 23 mm. pig embryo (series 67, slides 36-37 inclusive), $\times 50$. Sinistro-dorsal view.

5, right and left thoracic duct; veno-lymphatics have been completely replaced. Other explanations the same as above.

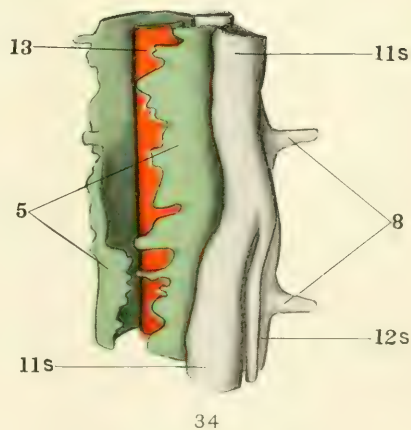
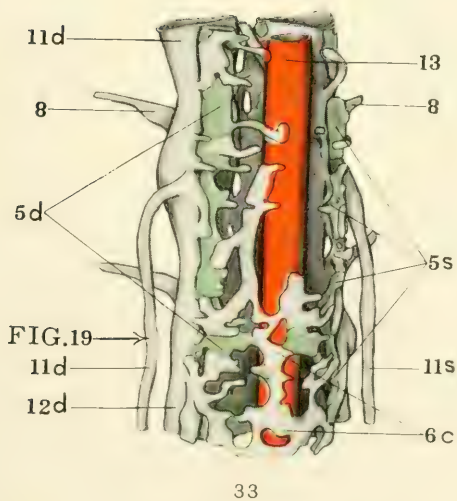
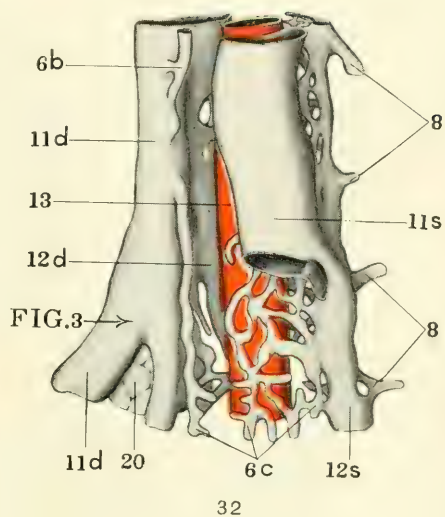
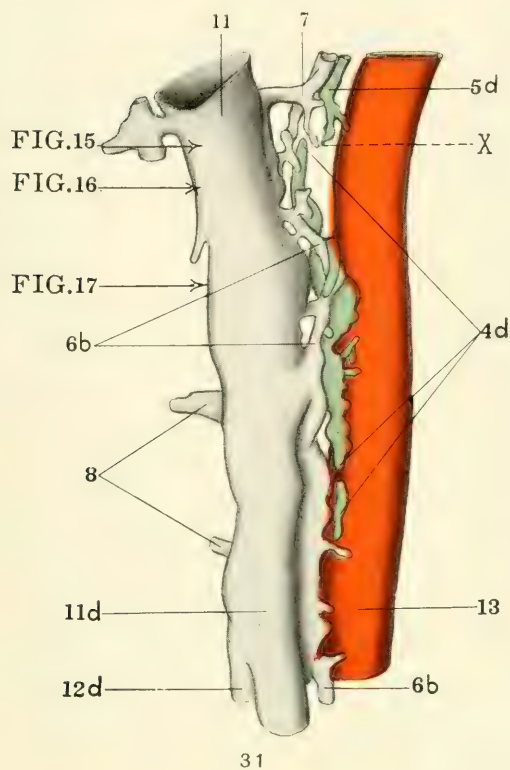


PLATE 5

EXPLANATION OF FIGURE

35 Reconstruction of the vascular channels of the lower cervical and thoracic regions in a 21.5 mm. pig embryo (series 192, slides 16-24 inclusive), $\times 50$. Sinistro-dorsal view.

- | | |
|----------------------------------------------------------------------------------------------|------------------------------------------------------------------|
| A, precardinal division | 11 <i>d</i> , 11 <i>s</i> , right and left postcardinal veins |
| B, postcardinal division | 12 <i>ls</i> , plexiform remnants of the left supracardinal line |
| 1, left jugular lymph sac | 13, aorta |
| 2, thoracic duct approach | 13 <i>b</i> , ductus arteriosus Botalli |
| 3, anterior lymphatic plexus or subsequent common trunk of the right and left thoracic ducts | 13 <i>d</i> , right subelavian artery |
| 4 <i>d</i> , 4 <i>s</i> , discontinuous thoracic ductanlagen | 13 <i>s</i> , left subelavian artery and branches |
| 5 <i>d</i> , 5 <i>s</i> , right and left thoracic ductanlagen continuous with lymph sac | 14, left sympathetic nerve trunk |
| X, X, extent of continuity in the ductanlagen connected with the lymph sac | 15, vagus |
| 7, spur or vestige of the former oblique vessel | 16, recurrent laryngeal nerve |
| 8, dorsal segmental veins | 17, oesophagus |
| 9, internal jugular vein | 18, trachea |
| 10, left precardinal vein | 21, left Cuvierian duct |
| | 22, left subelavian vein |
| | 23, cephalic vein |
| | 24, external jugular vein |
| | 26, subelavian approach of the jugular lymph sac |



THE FORM OF THE STOMACH IN HUMAN EMBRYOS WITH NOTES UPON THE NOMENCLATURE OF THE STOMACH

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TWELVE FIGURES

X-ray examinations of the stomach, in adults and especially in children, have led clinicians to inquire whether the stomach has a characteristic embryonic form which may sometimes persist. Figures of the typical embryonic stomach have, indeed, been published; but it must be remembered that the stomach changes in shape as the embryo grows older and, as Broman has found, its individual variations in embryos of the same stage of development is very great. Nevertheless certain fundamental subdivisions are strikingly distinct. These primary subdivisions, in which the embryologist is most interested, were keenly discussed by the early anatomists. In their writings many suggestive questions are raised, at the same time that the fundamental features of the organ are successively recognized and defined. In the following historical notes, taken from such works as are at hand, provisional definitions are offered for certain terms adopted at Basle but at present loosely employed, and attention is called to the features of the adult stomach which will be examined in the embryos.

The human stomach was first considered to be a simple sac with an orifice of entrance above and to the left, and an orifice of exit below and to the right. Vesalius (1543) in his figures designates the orifices as the 'superius ventriculi orificium' and 'inferius ventriculi orificium,' respectively. In his text, however, both are said to be placed superiorly, so that food shall not escape by its own weight, but when completely changed to chyme, shall

be propelled by force of the discharging stomach into the intestine. Fabricius ab Aquapendente (1618) likewise states that the *orificium inferius* is not inferior at all, and Spigelius (1627) places it in the highest part of the stomach; so that the term '*orificium dextrum*' was preferred, and finally the less objectionable Greek name '*pylorus*' (Latin, *janitor*), which had been introduced by Galen, became the accepted designation. Winslow, however, in 1732, insisted that the position of the orifices is such that "we ought with the ancient anatomists to call one of them superior, the other inferior."

The significance of '*cardia*' (Latin, *cor*), as applied to the oesophageal orifice, was discussed by Fabricius, who cites Galen as stating that the upper orifice of the stomach is called the heart because the symptoms to which it gives rise are similar to those which sometimes affect the heart, sometimes even the brain; but for Fabricius, *cardia*, as applied to this orifice, merely indicates a chief part of the body. Spigelius describes the *cardia* as consisting of circular fleshy fibers, so that the stomach, after having received food, may be closed perfectly, thus preventing fumes from rising, with consequent loss of heat. The *cardia* and *pylorus* are intimately associated with their respective sphincter muscles, but they do not include the adjacent portions of the stomach.

For the stomach as a whole these anatomists use the Latin '*ventriculus*,' rather than the Greek '*gaster*,' and the Latin term has been adopted at Basle. Since however, the adjective *gastricus* has been chosen instead of *ventricularis*, it seems desirable that *gaster* should be used in place of *ventriculus*, especially since *cardia* and *pylorus* are of Greek origin.¹

¹I am indebted to Prof. Albert A. Howard for the following note regarding these terms: *Gaster* is a Greek word meaning belly (the whole abdominal cavity) but was often used by the Greeks in the more restricted sense of stomach. It is not found in Latin with this meaning until very late (only after the literary period). *Ventriculus* is used quite consistently for stomach by Celsus and at times by Pliny the Elder. Cicero in one passage speaks of *ventriculus cordis*, but does not use *ventriculus* for stomach. If *gaster* is adopted I think the genitive *gastri* is preferable to *gasteris*, though as a matter of fact the genitive does not happen to occur in any Latin that is preserved to our time. Petronius has used the ablative plural *gastris* which would be the reason for deciding as I have.

The stomach, as described by Vesalius, is rounder and more spacious on the left side, and more slender on the right; to which Fabricius adds that it is not unlike a gourd with larger belly and narrower neck. On its dorsal side Vesalius found two swellings, separated by a vertical impression which was fitted against the trunks of the aorta and vena cava and the projecting bodies of the vertebrae. When the stomach was inflated, the impression and swellings were lost in an even rotundity. It was not until Willis (1674) described the pyloric antrum in the following passage, that a permanent subdivision of the stomach was established.

The other orifice, commonly called the pylorus, on the right side of the stomach, having a capacious and long, gradually narrowed antrum, ends in a small foramen and thence bent back is continued into the duodenum. Here the coats are much thicker than in any other part of the stomach.

Indeed the long and capacious antrum seems to be a sort of recess and diverticulum in the stomach, into which the more elaborated and perfected portion of the chylous mass may withdraw and there remain, while the other cruder and more recently ingested portion may be further digested in the fundus of the stomach (ed. of 1680, p. 13-14).²

Accompanying this description Willis published four lateral views of the stomach, with its coats successively removed. All of them show the antrum, but in a fifth figure, representing the everted stomach, its limits are most satisfactorily indicated (fig. 1). In this figure the antrum is shorter and broader than in one of the others, in which it has been stretched out so as to form a tube. In all of the figures it is clear that the antrum extends to the pylorus, which is referred to as its orifice.

Bidloo (1685) published a more accurate figure of the stomach, here reproduced as figure 2, but he failed to describe it adequately. He states that the base is provided with two swellings, *C* and *D*. In another figure, showing the same stomach partly laid open, the portion of the duodenum near the stomach (*A*) is labelled pylorus, but Bidloo does not refer in any way to the subdivision which in figure 2 has been labelled *B*. Cowper (1698), who republished

² For verifying and revising the Latin translations, the author is under obligation to Mr. S. R. Meaker.

Bidloo's plates, states that *A* is the part of the duodenum arising from the pylorus and adds that *B* is the antrum pylori.

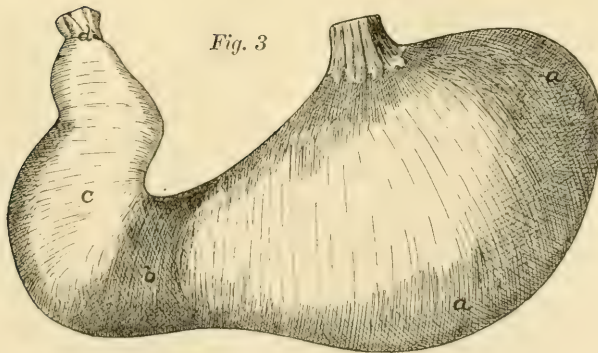
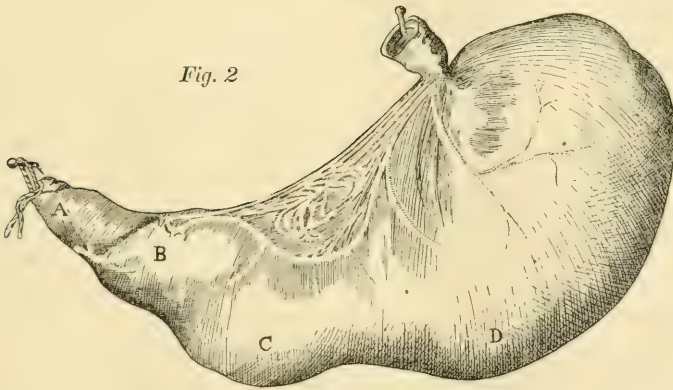
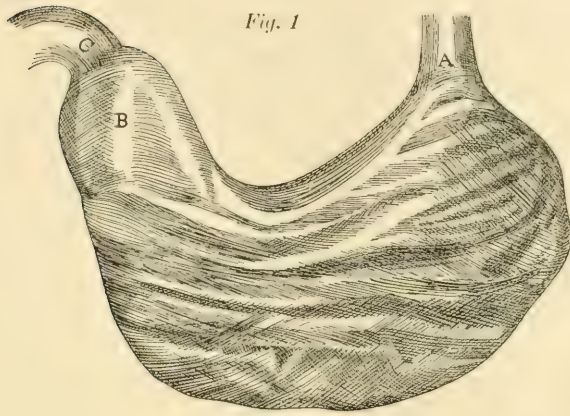
In 1732 Winslow described the large arch running along the greatest convexity of the stomach, and the small one directly opposite, and named them the great and small curvatures. Bichat (1802) states that "the great curvature ends simply at the pyloric orifice, without presenting anything of note unless it be the elbow (*le coude*) formed by this pyloric orifice, and named the small cul-de-sac; but there is no particular swelling at this place and the bend is precisely in the direction of the pylorus." Cloquet (1831) repeated this description and Cruveilhier (1834) made it more explicit. He states that at about 2 or 3 cm. from the pylorus "the stomach, bending sharply upon itself, forms a very pronounced elbow (*coude de l'estomac*) on the side of the greater curvature, and presents an ampulla, corresponding to an interior excavation, named by Willis the pyloric antrum, by others the small cul-de-sac."

As pointed out by Müller (1897), Cruveilhier was unjustified in identifying a pouch about an inch from the pylorus with the pyloric antrum of Willis; but he was correct in stating that "it is not rare to see a second ampulla beside the first, and a third but smaller one, on the side of the lesser curvature" (compare with figs. 2 and 3). These had not been recognized by Willis, but Cowper, in describing Bidloo's plate, was confronted with the question whether one or more of these parts was to be regarded as the antrum. In applying the term to the part adjacent to the

Fig. 1 Willis's figure of the inverted stomach re-drawn and reduced one-half. "A, Orificium sinistrum, sive os ventriculi. B, Pylori Antrum, in quo, Tunicæ crassiores existunt. C, Orificium ejus, cuo Duodenum annectitur."

Fig. 2 Bidloo's figure of the unopened stomach, re-drawn and reduced two-thirds, with lettering added from Bidloo's drawing of the same stomach opened, and from Cowper's edition of Bidloo's plates. A, pylorus (Bidloo); portion of the intestinum duodenum (Cowper). B, antrum pylori (Cowper). C, D, two bunchings out in the lower part or fundus of the stomach (Cowper); in fundo Gibbis ornatu duobus (Bidloo).

Fig. 3 Home's figure of "the human stomach inverted, to show the contraction which divides the cavity into two portions." Re-drawn and reduced two-thirds. aa, the cardiac portion. b, the contraction dividing the cardiac from the pyloric portion. c, the pyloric portion. d, the pylorus.



pylorus but not extending to the bend of the stomach, Cowper was justified by Willis's figure here reproduced as figure 1. According to Cunningham (1906) "no part of the organ is more definite and distinct" than the region which Cowper designated 'antrum pylori' and which, rediscovered by Jonnesco (1895), was named the pyloric canal. It may be defined as the part of the stomach adjacent to the pylorus, averaging 3 cm. in length, cylindrical when empty, bulbous when distended, separated from the remainder of the stomach by a groove on the greater curvature—the 'sulcus intermedius' of His (1903). For the small cul-de-sac of Cruveilhier the term 'pyloric vestibule' (Jonnesco, 1895) may be adopted.

Unfortunately Cowper's use of pyloric antrum has been overlooked by later anatomists, and the term has been so variously employed, as tabulated by Müller, that Müller, His and Cunningham have proposed to abandon it altogether. His has suggested an entirely new nomenclature for the pyloric region, as follows: for pyloric vestibule, *camera princeps*; for the swelling on the lesser curvature opposite the sulcus intermedius (fig. 3), *camera minor*; and for pyloric antrum, *camera tertia*. But these terms, as stated by Cunningham, are not in every respect satisfactory, and it may be well to retain the appropriate name 'pyloric antrum' in the sense of Cowper, following Meyer (1861) and Hasse and Strecker (1905).

The normal division of the entire stomach into two parts, cardiac and pyloric (of which the latter presents the subdivisions just described), was first recognized by Home (1814). He wrote as follows:

I found also, in the necessary examinations, that the dog's stomach, while digestion is going on, is divided by a muscular contraction into two portions; that next the cardia is the largest, and usually containing a quantity of liquid, in which there was some solid food; but the other, which extended to the pylorus, being filled entirely with half-digested food of an ordinary consistence. I shall, therefore, in my future description call that part which constitutes the first cavity the cardiac portion, and that which constitutes the second the pyloric portion (p. 140). The cardiac portion is in length two-thirds of the whole, but in capacity much greater (p. 139).

Home distinguished these two portions not only in the dog but, with varying distinctness and permanence, in many animals,

including the horse, pig, rat, rabbit and man. He did not describe or label the subdivisions of the pars pylorica, which however are clearly shown in his figure of the everted human stomach (fig. 3).

In connection with Home's work, the following more recent physiological observations are of interest. Schütz (1885) found that in the dog's stomach, contraction waves travel from the cardiac end to a place about 1 cm. from the beginning of the pyloric antrum (pars pylorica?), which in the isolated resting stomach may be recognized by a shallow annular constriction about 2 cm. behind the pylorus, and there end with a deep 'prae-antral constriction.' The second phase, which follows the first immediately, concerns the antrum only. The motions of the two parts may take place independently of one another. Moritz (1895) determined the pressure within the two parts of the stomach, and stated that the difference between them was greater than would be inferred from merely observing their motions. Cannon (1898) found that the stomach of the cat, as shown by X-ray examinations, is composed of two physiologically distinct parts—a 'busy antrum' and a cardiac reservoir. In 1911 he states that during normal digestion "slight constrictions appear near the middle of the body of the stomach, and pressing deeper into the greater curvature, course towards the pyloric end. When a wave sweeps round the bend into the vestibule, the indentation made by it increases." He adds that when vomiting occurs, a strong contraction at the angular incisure completely divides the gastric cavity into two parts. Thus the observations of Home have been amply confirmed and extended. Other X-ray observers, however, have considered that the antrum, or pars pylorica, of anatomists is merely the part of the stomach marked off by a passing peristaltic wave (Hertz; Kaestle, Rieder and Rosenthal; Barclay). In this they follow Sappey (1874), who was of the opinion that Home's subdivision of the stomach was based on fortuitous muscular contractions. This will be disproved by showing that the two divisions of the stomach are well marked in embryos in which the muscle-layers are still scarcely differentiated.

When a peristaltic wave remains fixed after death, the stomach may appear as "two joined together" (Riolan 1618), in which case the subdivisions need not correspond with the anatomical parts already described. Usually the constriction is near the middle of the stomach, and falls within the cardiac portion. Morgagni (1761) observed five cases, all in women. One of the stomachs was from a patient who had been troubled with excessive vomiting since birth, but none of the stomachs showed any sign of disease. Since several cases had been reported in men, Morgagni concluded that the double stomach was not a deformity due to stays, but had existed from the first formation of the organ. Sandifort (1777-1781), as quoted by Bettman, described a typical case in a fetus, the age of which is not stated in the citation. Delamare and Dieulafé (1906) reported a case in a new-born syphilitic infant, in which they describe an hypertrophy of the circular muscle at the place of constriction. The thickened muscle-layer may, however, be due to contraction, as indicated by the folded and thickened overlying layers shown in their figures. Cunningham (1906) holds that there is not an atom of evidence that the hour-glass stomach ever arises as a congenital deformity, but he is not prepared to state that the strictures which separate the two sacs of the bilocular stomach are always temporary and fleeting.

The change in the direction of the lesser curvature is more dependable as a boundary between the pars pylorica and pars cardiaca, than the constriction which is present in certain cases but "not as a rule" (Huschke). The lesser curvature, which is concave along the cardiac portion, becomes convex along the pars pylorica (Meckel 1820; Huschke 1844). Retzius (1857) figured a deep stricture in the lesser curvature at the beginning of the bulbous pars pylorica, where Luschka (1869) frequently found an acute angle directed toward the gastric cavity. This notch has been named by His (1903) the 'incisura angularis,' and it occurs between the two parts of the stomach. Along the greater curvature the boundary is less clearly marked. It is indicated by the change in direction already described as the elbow of the stomach, and referred to by Home as "an angle formed at the part where the temporary contraction takes place." Some-

times the constriction is slightly to the cardiac side of the elbow, as shown in figure 3.

The angle which separates the two parts of the stomach is obscure in the older drawings in which the organ is almost horizontally placed. According to Bichat (1802) "when the stomach is filled its obliquity increases considerably; often it appears almost perpendicular, so that the right extremity . . . is strongly recurved upward, and forms a very acute angle with the body of the organ." Luschka (1869) similarly found that the greater part of the stomach, as a rule, has a precisely vertical position, but that the pars pylorica is directed almost transversely. Both of these forms, with vertical body and transverse or ascending pars pylorica, will be seen in the embryos to be examined.

The pars pylorica and its subdivisions having been described, the pars cardiaca may next be examined. It is divided into the 'saccus caecus,' now called the 'fundus;' the 'corpus' or body; and the gastric canal. The term fundus was appropriately applied by Vesalius to the lower part of the stomach, which in the transverse position of the organ, extends well toward the pyloric region. It was so used by Willis (1674); and by Cowper (1737), as seen in figure 2. Caldani (1804) makes fundus synonymous with greater curvature. The bulging left or upper extremity of the stomach received the special name 'saccus caecus' (Haller, 1764; Caldani, 1804). But Meckel (1820) considered fundus and saccus caecus as synonyms, and preferred fundus; Huschke (1844) likewise made them synonymous, but adopted saccus caecus, which Henle used in 1866. Nevertheless, fundus has become adopted for the highest part of the stomach and saccus caecus has been rejected. The fundus lies at the left of the cardia, being separated from the oesophagus by a notch, the 'incisura cardiaca' of His (1903). Below, as described by Cloquet, the fundus terminates almost imperceptibly in the greater curvature. It is therefore bounded arbitrarily by a horizontal plane at the level of the inferior border of the cardia (Jonnescio), or by a line prolonging the axis of the abdominal part of the oesophagus (Keith and Jones, 1902).

According to Keith and Jones the fundus arises in human embryos as a localized outgrowth or diverticulum of the stomach,

and in its manner of origin has much in common with the caecum and vermiform process. From numerous observations they conclude that "it is not uncommon to find in the stomach of the anthropoids, and to a lesser degree in that of the apes (especially in *Mycetes*) clear indications of three chambers, namely, a fundus, a body, and a pyloric part; and that therefore the stomach of the Primates (excluding the Lemuroidea) is probably tripartite in nature." It should be noted that the fundus as defined by Keith and Jones is a larger part of the stomach than that set off by Jonnesco, and that their boundary is justified by comparison with the stomach of *Semnopithecus* which they have figured. If the fundus corresponds in any way to a first stomach or rumen, it may be regarded as the globular upper end of the organ which is often marked off by the contraction of the corpus.

The body of the stomach (*corpus gastrici*), as defined by Rüdinger (1873), is its middle subdivision, situated between the fundus and the *pars pylorica*. Froriep (1907) proposed to rename it the *pars intermedia*; but since it is a portion of the *pars cardiaca*, and is not intermediate between the *pars cardiaca* and *pars pylorica*, the proposed term would lead to confusion. Jonnesco (1895) defined the body as including the pyloric vestibule, but in the same paragraph he described the boundary between the vestibule and "*le corps proprement dit.*" Müller (1897) included the fundus with the body, making *corpus* and *pars cardiaca* synonymous. It is only by accepting Rüdinger's earlier definition that *corpus* becomes a useful term. The *corpus* may be contracted at any point, as in the hour-glass stomach, in which case part of it appears to belong with the fundus and the remainder with the *pars pylorica*. Sometimes it is contracted as a whole, but more often it is relaxed, and its boundaries are then ill-defined.

The gastric canal is a channel which follows the lesser curvature, appearing as a groove when seen from the inside of the stomach. It suggests a continuation of the oesophagus, split open toward the gastric cavity, and has been named the *sulcus oesophageus*, *sulcus gastricus*, *sulcus salivalis* and *canalis salivalis*. It is confusing, however, to refer to this channel as a *sulcus*, since the

external grooves of the stomach are so designated (*sulcus intermedius*, *sulcus pyloricus*), and it is undesirable to name a part of the stomach oesophageal or salival. Therefore the term gastric canal, '*canalis gastricus*,' is here proposed, and *canalis* is used as in Latin for an open canal, which in this case may become a tube during its physiological activity, by the approximation of its lips.

The gastric canal has long been known in ruminants, but in its less highly developed condition in the human stomach, it has attracted little attention. In man it is generally supposed to be due to the arrangement of the oblique muscle fibers, which were first described by Willis (1674), in connection with a figure of the stomach in the position shown in figure 1. The 'top' of the stomach is accordingly toward the lesser curvature, and the 'fundus' is toward the greater curvature. Willis wrote as follows:

These muscle fibers, which are seen to arise behind the cardia and to pass around its left margin, are carried forward to the right portion of the stomach. A notable bundle of them, proceeding in straight lines along the top of the stomach on either side, encounters the antrum, and spreading over the length of its cavity in a scattered manner, terminates in the pylorus. Moreover the remaining fibers of this layer extend obliquely over the walls of the stomach on both sides, and then directly toward the fundus where they come together. The function of the former (the straight bundles) seems to be to bring one orifice toward the other in emptying, by making them lower and higher respectively (ed. of 1680, pp. 11-12).

Retzius called attention to this description by Willis and, as reported by Gyllenskoeld (1862), he supplemented it as follows:

The upper portion of the oblique fibers of the human stomach serves to form a sort of trough along the lesser curvature which, under the control of the motor nerves, becomes more or less closed; along this path possibly fluids and soft things, saliva, etc., may proceed directly from the oesophagus to the *pars pylorica*, passing by the cardiac portion, which corresponds to the first two stomachs of ruminants and the non-glandular part of the stomach in rats.

The correctness of this conjecture concerning the passage of fluids was established by Cohnheim (1908), who was surprised

to find that water or salt-solution passed rapidly through the full stomach of a dog, without mixing with the gastric contents.

Gyllenskoeld (1862) states that the oblique fibers extend only to the pars pylorica, and not to the pylorus as described by Willis. This has been confirmed by Kaufmann (1907). He found that there is no sphincter of circular fibers separating the pars cardiaca from the pars pylorica, but that the furrow between them has a special structure, since it is the place where the oblique fibers terminate and interlock with the circular fibers.

Hasse and Strecker (1905) have named the folds which bound the gastric canal the 'plica hepatica' and 'plica aortica' respectively, and state that they are connected with one another by the 'plica cardiaca' which passes around the cardia, projecting into the stomach beneath the incisura cardiaca. According to Hasse and Strecker the plica cardiaca does not form a valve for the cardia, as Braune (1875) thought possible from the result of experiments on a cadaver. The hepatic, cardiac and aortic plicae together form a U-shaped structure, across the open end of which is the 'plica angularis.' This is beneath the incisura angularis, at the beginning of the pars pylorica.

Waldeyer, who describes the channel from cardia to pars pylorica as the 'Magenstrasse' (1908), considers that its formation depends upon the oblique muscles, rather than upon folds which arise in relation with adjacent organs. In the following pages evidence will be offered to show that the gastric canal is a distinct epithelial structure, arising independently both of the muscle and the surrounding organs.

There remain to be considered two structures which are beyond the limits of the stomach—the 'antrum duodenale' and the 'antrum cardiacum.'

Retzius (1857) states that the beginning of the duodenum is often specially rounded, not only in man, but in a large proportion of mammals; in dolphins it has been considered a part of the stomach. Owen (1868) remarks that in all Artiodactyles the duodenum is dilated at its commencement; it there forms a distinct pouch in the camel. For this pouch Retzius proposed the

names "antrum or atrium duodeni" but used the former in his figures. Luschka (1863) refers to a flask-shaped expansion at the beginning of the duodenum, which in his figure is called the 'antrum duodenale.' This structure will be seen to be far more distinct in human embryos than it appears to be in adults.

The cardiac antrum was first described by Luschka (1863) as follows:

At the junction of fundus and lesser curvature the oesophagus enters the stomach, forming a funnel-shaped expansion—the cardia. Although ordinarily the cardia is continued into the rest of the stomach without definite boundary, in rare cases the funnel-like expansion is sharply marked off by an external depression and corresponding internal elevation, thus forming a sort of cardiac antrum (p. 179).

In 1869 Luschka adds that if this funnel is to be regarded as part of the stomach, the beginning of which is not rather to be considered at the base of the funnel where the stratified epithelium ends in a zig-zag line (fig. 3), "then the funnel-shaped expansion must be specially designated as the *pars cardiaca*."

Thus Luschka proposed two names for a single structure; first, cardiac antrum; and later, in case the antrum is to be regarded as part of the stomach, *pars cardiaca*. The latter may be rejected, since it is generally agreed that the cardia is at the base of the cone, and that therefore 'cardiac antrum' is "merely another name for the intra-abdominal part of the oesophagus" (Cunningham). Moreover the earlier use of *pars cardiaca*, or cardiac portion, for the fundus and corpus taken together, was overlooked by Luschka, and by certain later anatomists who have proposed to substitute *Hauptmagen* (His), *saccus ventriculi* (Hasse and Strecker) and *pars digestoria* (Froriep).

The fundamental subdivisions of the stomach and adjacent parts of the digestive tube, as they have been defined in the preceding pages, are presented in figure 4 and in the following table, with authority for certain of the definitions adopted:

Antrum cardiacum (Luschka, 1863)

Gaster

Cardia

Pars cardiaca gastræ (Home 1814)

Fundus (Meckel 1820)

Corpus (Rüdinger 1873)

Canalis gastricus

Pars pylorica gastræ (Home 1814)

Vestibulum pyloricum (Jonnescio 1895)

Antrum pyloricum (Willis 1674 (?); Cowper 1698)

Pylorus

Antrum duodenale (Retzius, 1857)

As boundaries between these parts, the following may be recognized: Between the cardiac antrum and fundus, the 'incisura cardiaca;' between cardiac and pyloric parts, the 'incisura angularis;' between pyloric antrum and pyloric vestibule, the 'sulcus intermedius' (all of His 1903); at the pylorus, the 'sulcus pyloricus' (Luschka 1863).

THE STOMACH IN HUMAN EMBRYOS

The embryonic stomachs to be examined are five in number, from embryos between 10 mm. and 45 mm. in length. Thus they are all smaller than the specimens studied by Müller, but similar stages have been described by Broman in his extensive work on the omental bursa. Broman modelled not only the gastric epithelium, but also entire stomachs, including the mesodermal portion. In the models to be described, only the epithelium has been included, since this is the portion having characteristic shape, to which the other layers subsequently conform.

In the youngest embryo (10 mm., fig. 5) the stomach is no longer a simple sac with superior and inferior orifices, but is already divided into an expanded pars cardiaca and a tubular pars pylorica. Between the two, and almost exactly in the middle of the stomach, is the incisura angularis. Since the incisure in the adult is perhaps twice as far from the cardia as from the pylorus, it is evident that the pars pylorica is relatively long in early stages. This is strikingly shown in other models of the series (figs. 6-9).

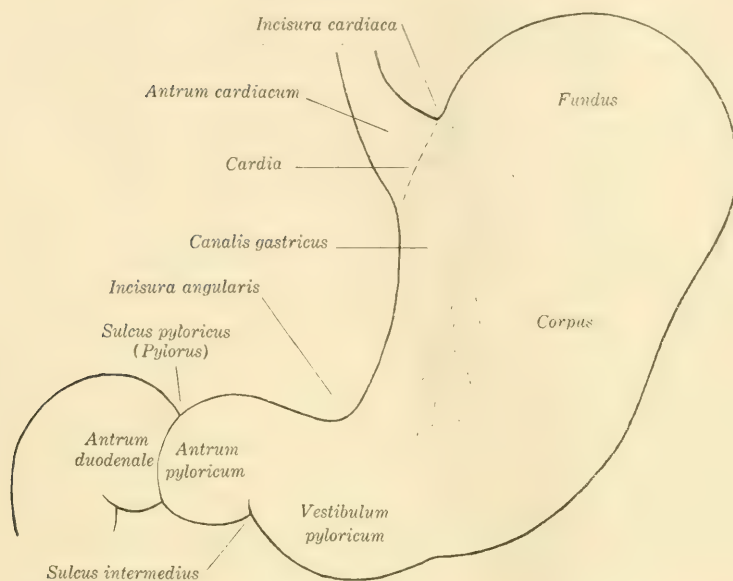


Fig. 4 Diagram showing the subdivisions of the human stomach

It is true also in the cat, if one may judge by comparing Thyng's model of the stomach of a 10.7-mm. embryo (this Journal, vol. 7, p. 496) with Cannon's tracings from the adult. In ruminants a constriction early separates the rumen and reticulum from the psalterium and abomasum; according to Ellenberger and Baum the abomasum is larger than the rumen in embryos and very young animals, but later this relation is reversed. The relatively large size of the pars pylorica in early stages is therefore not limited to human embryos.

The cardia cannot be definitely located in the 10-mm. embryo (fig. 5) since the oesophagus, in joining the stomach, expands into a flattened cone, one margin of which extends to the angular incisure. A similar extension of the oesophageal cone to the incisure is clearly seen in Broman's model of the stomach of the seventh embryo in his series (11.7 mm.). At 16 mm. (fig. 6) the body of the stomach may be recognized along the lesser curvature, separating the oesophageal cone from the angular incisure; but a canal, distinctly marked out above and indicated below,

passes along this curvature from the oesophagus to the pars pylorica. A more distinct canal in this position is seen in two of Broman's models, from embryos of 10 mm. and 16.2 mm. respectively. Apparently this canal has not been previously described in embryos, although Toldt (1879), referring to the general direction of the oesophagus in a 23-mm. specimen, states that it descends into the stomach "in such a way that the lesser curvature forms, as it were, a continuation of the ventral border of the oesophagus."

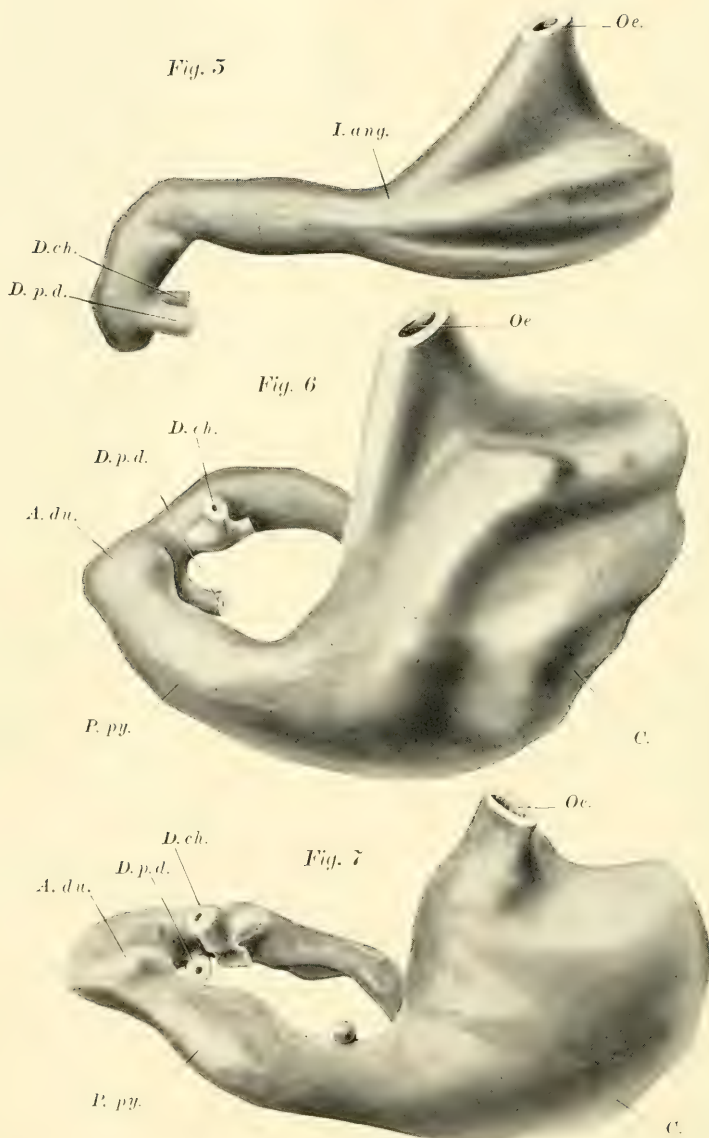
In the embryos of 19.3 mm. and 19.0 mm. shown in figures 7 and 8 respectively, the canal is not seen. The first of these stomachs is abnormal, but the second specimen is unobjectionable. Moreover in Broman's figure of the stomach from an embryo of 21 mm., there is no trace of the channel. Its obliteration, if normal, appears to be temporary however, for in the 44.3-mm. specimen shown in figure 9, it is more distinct than in preceding stages. It passes from the conical cardiac antrum to the angular incisure. This embryo, owing to its large size, was not perfectly preserved, and the epithelium has separated from the mesenchyma; but whether one, or the other, or both of these tissues has shrunk is uncertain. The model may, however, be accepted as giving an essentially correct idea of the shape of the stomach, since the separated mesenchyma presents corresponding ridges and furrows. The distinctness of the gastric canal is strikingly shown when the model is viewed from the inside (fig. 10). It takes a slightly S-shaped course from the stellate cardia to the orifice of the pars pylorica, and is bounded by a rounded plica aortica, and a more prominent and angular plica hepatica. These folds are not formed, as described in the adult by Hasse and Strecker, through compression of the border of the stomach between the aorta behind and the caudate lobe of the liver in front; for the outer layers of the stomach are not indented. Moreover at this stage there are no bands of oblique fibers to account for the canal. If the channel proves to be a constant feature of embryos of this stage, and it is present in an embryo of 37 mm. which was not modelled, it may be that the arrangement of the oblique fibers is a consequence rather than the cause of the gastric canal.

In the same way that the gastric canal accords with the 'oesophageal sulcus' of ruminants, which is described by comparative anatomists as a continuation of the oesophagus open on one side, the cardiac antrum may correspond to the 'atrium ventriculi.' This, according to Ellenberger and Baum is "a dome-shaped swelling on the dorsal side of the reticulum and thoracic end of the rumen, which is only indistinctly marked off from them by a shallow groove; ventrally its cavity passes directly into that of the reticulum, and caudo-ventrally into the vestibule of the rumen; toward the thorax it rests against the diaphragm near the hiatus oesophageus." From the general 'atrium' seen in figure 5, the lower part is set off as the gastric canal, and the upper part remains as the cardiac antrum (figs. 8 and 9). From studies of the adult stomach it may be assumed that the cardia is at the base of this antrum, which therefore belongs with the oesophagus.

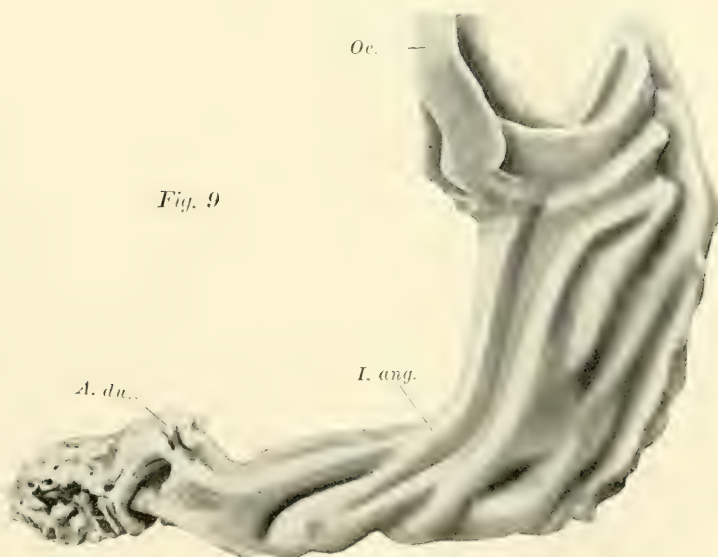
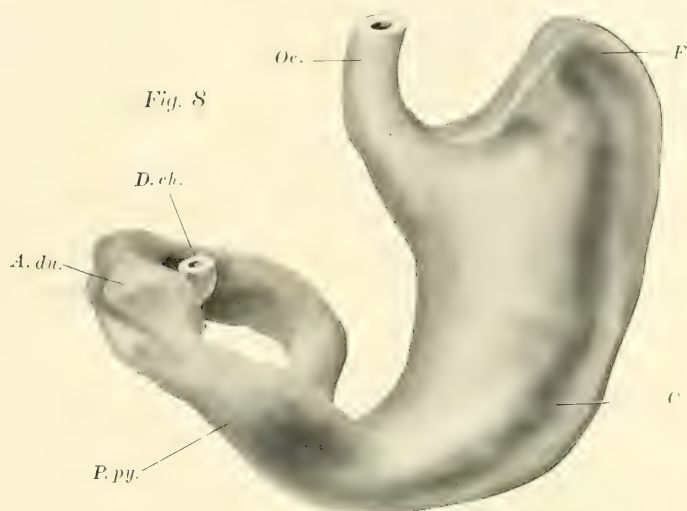
The development of the fundus of the stomach has been described by Broman (1911) as follows:

By the beginning of the second month the cranial part of the greater curvature begins to bulge out. But not until the third month, or later, is this outpocketing generally directed so strongly cranial that its blind end comes to lie above the orifice of the oesophagus. Only from this time, therefore, can we speak of a distinct gastric fundus (pp. 326-328).

Similarly Keith and Jones state that the outgrowth is best marked in embryos of the third and fourth month. But as shown in figures 8 and 9, and by the fact that Toldt, in an embryo of 48 mm., found a well marked fundus projecting toward the concavity of the diaphragm, it is clear that the fundus may be well developed in the second month. In the model shown in figure 9, the fundus when seen from above, presents a curious appearance, since seven prominent ridges converge toward its apex. Two of them come from the cardiac antrum, sweeping in a semicircular curve beneath the cardiac incisure, thus resembling the ridges seen in figure 8. There is normally no boundary between the fundus and corpus, but in an abnormal embryo of 18.5 mm., described by Broman, the fundus is cut off by a rather deep constriction. Broman states that this specimen suggests an hour-glass stomach, from which, however, it is essentially different, since the oesopha-



Figs. 5 to 9 Models of the gastric epithelium in human embryos, as follows: figure 5, 10 mm., Harvard Embryological Collection, Series 1000, $\times 50$ diam.; figure 6, 16.0 mm., H. E. C. 1322, $\times 35$ diam.; figure 7, 19.3 mm., H. E. C. 1597, $\times 30$ diam.; figure 8, 19.0 mm., H. E. C. 819, $\times 26$ diam.; figure 9, 44.3 mm., H.



E. C. 1611, $\times 18$ diam. *A. du.*, antrum duodenale. *C.*, corpus gastrici. *D. ch.*, ductus choledochus. *D. p. d.*, ductus pancreatis dorsalis. *F.*, fundus gastrici. *I. ang.*, incisura angularis. *Oc.*, oesophagus. *P. py.*, pars pylorica gastrici.

gus enters the part toward the pylorus. The fundus is best marked when the pars cardiaca is in an approximately vertical position, and this is the case in figures 6 to 9. Broman, however, has found a greater variety of positions. In an embryo of 21 mm. he figures the stomach as horizontal, so that both orifices are superior, as described in the adult by Vesalius; but this position must be regarded as exceptional.

The body of the stomach requires no comment other than that its ridges appear to be rather definitely placed. The shelf-like

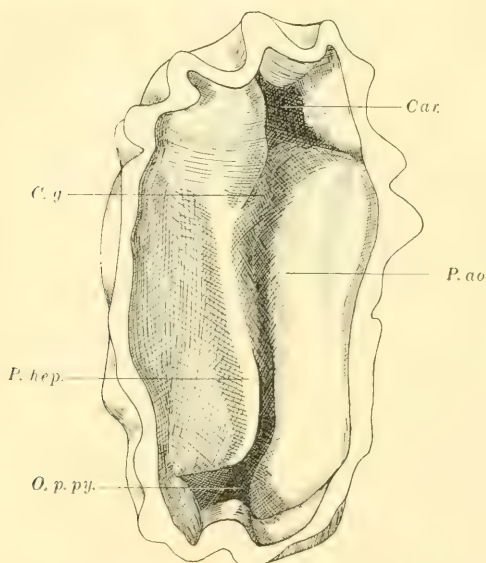


Fig. 10 Model of the interior of the stomach, from an embryo of 44.3 mm., H. E. C. 1611, $\times 25$ diam. *Car.*, cardia. *C.g.*, canalis gastricus. *O.p.py.*, orificium partis pyloricae. *P.a.*, plica aortica. *P.hep.*, plica hepatica.

prominence at the base of the oesophageal cone in figure 5, is evidently represented by the chief fold which extends horizontally across the base of the fundus, and bends down parallel with the lesser curvature in figure 6. Such an angular fold (with a subdividing furrow) is seen in figure 9, and it is clearly shown in embryos of 10 and 16.2 mm. figured by Broman. Why the ridges are absent from other specimens, as in figures 7 and 8, and in several of Broman's embryos, is not apparent.

The position of the pylorus could not be determined with certainty in the 10-mm. embryo (fig. 5); and Tandler (1900) states that in an embryo of 11 mm. the pylorus is not marked. At 14.5 mm., "where the stomach passes into the duodenum, therefore at the place of the future pylorus" he saw "a considerable thickening of the epithelium." The epithelial proliferation, which Tandler describes, is seen throughout the upper part of the duodenum. It is not evident that he recognized the local swelling, chiefly on the upper side of the digestive tube, which is

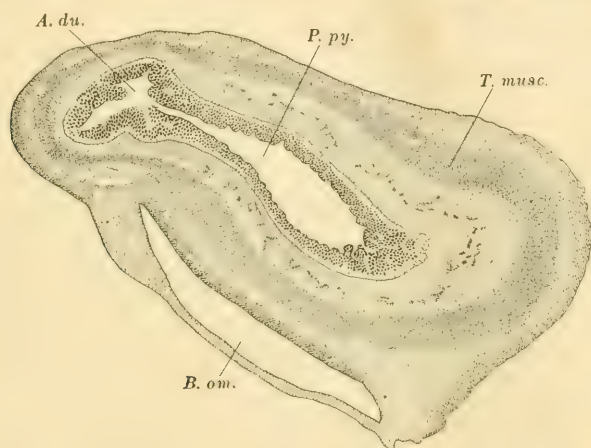


Fig. 11. Frontal section through the pylorus of a 19-mm. embryo, H. E. C. 828, section 330, $\times 40$ diam. *A. du.*, antrum duodenale. *B. om.*, bursa omentalis. *P. py.*, pars pylorica. *T. musc.*, tunica muscularis.

shown in figures 6 to 8. This swelling, which distinctly marks the position of the pylorus when the muscle-layers are still undifferentiated, and scarcely to be recognized, is apparently the duodenal antrum of Retzius. In a frontal section through the pars pylorica of a 19-mm. embryo, it appears as shown in figure 11. At this stage the musculature of the pars pylorica is considerably thicker than that of the duodenum, but in this it conforms to the shape of the epithelial tube. In figure 9 the duodenal antrum is seen to be smoother than the more distal part of the duodenum, recalling the statement of Retzius that here, in the adult, the valvulae are absent and the villi are short. In this embryo the

gastric epithelium is seen to be slightly invaginated into the duodenal tube, as observed by Cunningham at birth. Toldt found that the sulcus pyloricus could be seen externally in an embryo of 48 mm., and presumably it could have been found in this specimen by dissection.

The pars pylorica, even in the 44.3-mm. embryo, fails to show distinct subdivision into antrum and vestibule. Müller, who studied dissections of the embryonic stomach, states that in the 'first fetal period' the pyloric antrum is a direct continuation of the pyloric vestibule, but that later, when the pars pylorica is bent convexly upward, the general direction of the vestibule is upward, and of the antrum, downward. In the earlier period the antrum is characterized by "its cylindrical form and the great development of its muscle-layer." In the still earlier stages under discussion, neither distinction is applicable, for the entire pars pylorica is cylindrical, and as shown in figure 11, its musculature is thick. It is possible that the short and relatively smooth terminal portion of the pars pylorica, which in figure 9 is seen to be directed upward, represents the antrum; but this cannot be affirmed without further investigation.

In conclusion, the abnormal stomach shown in figure 7 may be considered. It is of special interest since Gardiner (1907) has described the stomach of a child of three months, which presents a very similar condition. In the embryo there is a round nodule of epithelial cells near the angular incisure. In sections (figure 12) this nodule appears as a compact ring of radiating cells arranged about a lumen. Toward the gastric epithelium there is one section in which this structure fails to appear, so that it is apparently detached, but a short stem projects towards it from the adjacent epithelium. Both the nodule and its stalk are inside of the muscular coat. A comparable but larger structure was found by Lewis and Thyng in the duodenal region of a 20-mm. pig (figured in this Journal, vol. 7, p. 509). In that case, however, the detached portion, which had become cystic, lay outside of the tunica muscularis. That the nodule in the human embryo is an accessory pancreas, is made certain by Gardiner's specimen, in which a well developed gland with typical islands occurs in a

corresponding position. Similar epithelial nodules were frequently found by Lewis and Thyng in young pig embryos, but they hesitated to interpret them as pancreases because of their abundance, and because they were never seen to branch like true pancreases. They may, however, as Elze has shown, be distinguished from the epithelial pockets of the gall-bladder and

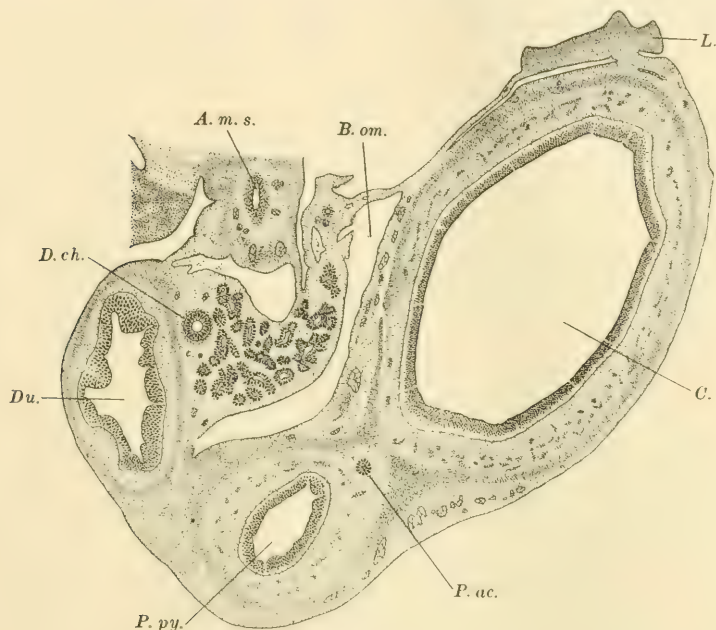


Fig. 12. Section through an abnormal stomach of an embryo of 19.3 mm., H. E. C. 1597, section 730, $\times 35$ diam. *A.m.s.*, arteria mesenterica superior. *B.om.*, bursa omentalis. *C.*, corpus gastrici. *D.ch.*, ductus choledochus. *Du.*, duodenum. *L.*, lien. *P.ac.*, pancreas accessorium. *P.py.*, pars pylorica gastrici.

small intestine which these authors described, and which seem to be transient irregularities of the expanding tubes. Accepting the small, round, compact nodules as accessory pancreases, we may conclude that they arise at about the time when the normal pancreases become established, and usually at no great distance from them, either up or down the intestine. Subsequent elongation of the tube may carry them farther away. They may be

assumed to develop slowly, since in the early stages they fail to produce branches like the adjacent normal pancreases; and as they are frequently seen to be detached, probably many of them degenerate without becoming functional glands.

Taken as a whole the stomach which Gardiner described is shaped like a retort. It has a globular cardiac end, 7 to 8 cm. in diameter; 'a constriction about its middle;' and a tubular pyloric portion, 3 to 4 cm. in diameter. If the cardiac half of the stomach shown in figure 7 should be pressed down, so that the lesser curvature became horizontal and the pars pylorica seemed to leave the upper portion of the corpus, then the form shown in Gardiner's case would be duplicated. Although Gardiner describes his case as an hour-glass stomach, it should not be classed with those which are due to muscular contraction. It is an arrest of development, in which the pars pylorica remains clearly set off from the pars cardiaca, and as in the 19.3-mm. embryo, the line of separation is in the middle of the stomach.

CONCLUSIONS

In addition to suggestions in regard to the nomenclature of the stomach, presented in tabular form on p. 490, the following conclusions may be drawn.

In the stomachs of embryos from 10 to 45 mm. in length, the division into pars cardiaca and pars pylorica is well marked; the latter is relatively long, constituting one-half the length of the stomach.

The oesophagus in joining the stomach in 10-mm. embryos forms a cone extending to the angular incisure. Later this cone gives rise to the cardiac antrum above, and to a downward prolongation of the antrum below. This prolongation, which extends along the lesser curvature, constitutes the gastric canal (*canalis gastricus*). It was found to be well developed in an embryo of 44.3 mm.

The fundus develops during the second month as a conical pouch; its boundary toward the corpus is arbitrary.

The position of the pylorus is first indicated by the antrum duodenale. The pylorus, like the gastric canal, is primarily an epithelial differentiation, to which the musculature conforms.

The occurrence of an accessory pancreas near the angular incisure is shown in an embryo of 19.3 mm., in connection with a stomach which would probably have presented a permanent stricture between the pars cardiaca and the pars pylorica, thus giving rise to one form of the so-called hour-glass stomach.

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